Effect of substrate type on diatom-based water quality assessments in the Mount Lofty Ranges, South Australia

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Abstract Diatoms have been widely used in stream health assessments as they are highly sensitive to water quality. There is no standardised method for diatom-based stream health assessments causing many substrates, both natural and artificial, to be used. Few studies have investigated the impact of substrate type on diatom assemblages in streams with highly variable water levels. To determine if the substrate type affects these assessments, diatoms were analysed from an artificial substrate (rope), rock and mud, from 17 sites in the Mount Lofty Ranges, South Australia. Rope has been advocated as a substrate as it mimics aquatic plants, while having advantages of artificial substrates (e.g. standardised habitat). We found that substrate

type did not affect the stream health assessment based on the Diatom Species Index for Australian Rivers and hence the additional cost of deploying ropes in such studies is unlikely to be warranted. However, substrate type influenced diatom–nutrient relationships, with no relationship between nutrients in the stream water and diatoms in the mud substrate—possibly due to the nutrient subsidy provided by mud. Therefore, by contrast, the absence of nutrients provided by the rope substrate indicates it is suited to studies of nutrient status and sensitivity in streams.

Keywords Bioassessment · Streams · Attached algae · Benthos · Fresh waters · Ecosystem

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Introduction

Accurately assessing stream water quality is important as water quality impacts biodiversity, stream-based recreation and commerce, and the quality of human drinking water supplies (Ndaruga, 2004; World Health Organisation, 2011). However, adequately assessing stream water quality is difficult since chemical “spot” sampling can be unrepresentative of the full range of variability, especially for temporary and ephemeral streams that regularly cease to flow for extended periods (Allan et al., 2006; Madrid & Zayas, 2007). These streams are common in areas with semi-arid climate such as South Australia (Botwe et al., 2015). Biological assessment methods can address this problem because they integrate responses to physical and chemical conditions over time periods of weeks to years (Norris & Morris, 1995; Schofield & Davies, 1996; Stevenson et al., 2010). Ideally, water quality assessments utilise a range of indicators that themselves are targeted towards assessing the specific values of streams that management seeks to protect (Bunn et al., 2010). As stream water quality is influenced by multiple chemical and physical factors—such as nutrient concentrations, pH, salinity, bed rock type, climate and flow regime—a range of bio-indicators are useful to help identify significant changes or thresholds that may be used for setting water quality guidelines (Boulton, 1999; Karr, 1999; Resh, 2008; Tibby et al., 2019). However, in resource-limited situations, often one or a small number of indicators is the focus. Hence, it is important to understand the efficacy of individual assessment methods in assessing stream health. Macroinvertebrate assessments dominate the literature in Australia (Tibby et al., 2019). However, there has been a marked expansion of diatom-based stream assessment, particularly outside the temperate zone (e.g. Oeding & Taffs, 2015, 2017; Tan et al., 2017; Tsoi et al., 2017; Mangadze et al., 2019). Macroinvertebrates are particularly good indicators of habitat quality, while diatoms may better reflect the physical and chemical composition of water quality (Newall et al., 2006; Resh, 2008; Gabel et al., 2012).

Diatoms are a group of aquatic algae that are highly sensitive to a variety of water quality parameters (in particular pH, salinity and nutrients) and, as diatoms have short life spans, they generally reflect recent water quality (i.e. in the weeks before sampling;

Stevenson et al., 2010), rather than past conditions, as may be the case for longer-lived organisms (e.g. fish and many macroinvertebrates). Consequently, diatoms have been used extensively as indicators in water quality assessments (e.g. Kelly et al., 1998; Potapova & Charles, 2002; Hering et al., 2006; Philibert et al., 2006; Smucker & Vis, 2010). Yet despite the extensive use of diatoms in stream water quality research, there is no standard sampling method applied, with numerous different approaches being used (Round, 1991; Kelly et al., 1998; Lane et al., 2003). Standards such as European Standards EN 13946 and EN 15708 advocate the use of a range of natural and artificial substrates including rock, mud, sand, plants, glass slides, brick and tiles for sampling diatoms (e.g. Tuchman & Stevenson, 1980; Round, 1991; Lane et al., 2003; Cantonati & Spitale, 2009). However, for seasonal streams, these artificial substrates may not be adequate due to highly variable water levels affecting the substrate's distance from the top of the wetter channel.

The influence of different substrates on diatom composition has been shown to vary. Diatom traits such as their growth form (motile, low vs. high profile) and strength of attachment can influence the relationship between water quality and community structure in streams (Passy, 2007; Berthon et al., 2011; B-Béres et al., 2014, 2016; Lukács et al., 2018). Some studies have found that particular diatom species are better adapted to certain microhabitats and thus preferentially colonise specific substrate types (Stevenson & Hashim, 1989; Round, 1991; Potapova & Charles, 2005; Cantonati & Spitale, 2009; Dalu et al., 2014, 2016). However, other studies find no significant difference in diatom assemblages between different natural substrates and between natural and artificial substrates (Cattaneo & Amireault, 1992; Jüttner et al., 1996; Townsend & Gell, 2005; Bere & Tundisi, 2011; Wojtal & Sobczyk, 2012). It may further be the case that although the composition of taxa can vary between substrates this may not affect diatom-based assessments of water quality (such as those based on indices).

Artificial substrates are advantageous in water quality assessments since they provide uniform habitat conditions, are exposed to water quality over known periods of time and can be deployed at all sites of interest. The latter is in contrast to natural substrates such as rock and mud which are unlikely to be found in

all lowland or upland stream sites, respectively (Round, 1991; Goldsmith, 1996). Rope has been recommended as an artificial substrate for diatom collection in several protocols (Kelly et al., 1998; Karthick et al., 2010). Rope is robust (in contrast to glass slides), has less variation in micromorphology (vs. bricks and tiles) and has low nutrient concentrations relative to other substrates such as mud and plants (Goldsmith, 2000; Karthick et al., 2010). Moreover, frayed rope has properties similar to macrophytes in that it can float on the stream surface and has a branching structure that supports high species diversity (Goldsmith, 2000). This allows rope to remain at the same position in the water column even with high variability in stream water levels. As a result, diatom communities that develop on rope may be similar to natural diatom communities (Goldsmith, 2000; Karthick et al., 2010). Given these advantages, Tibby et al. (2019) recently used diatom communities on ropes in the Australian state of South Australia to suggest that stream water guidelines in the state are not sufficiently stringent. However, to date, the efficacy of using rope has not been tested. Moreover, since the use of artificial substrates requires two field exercises (one for deployment and one for collection), any benefits must be assessed in the context of the additional opportunity costs.

This study aims to assess (1) if the diatom species composition and diversity differ significantly between mud, rock and rope substrates, (2) how the diatom species composition on each substrate responds to environmental gradients, and (3) whether (any) differences between the substrates affect stream health assessment using the Diatom Species Index for Australian Rivers (DSIAR) (Chessman et al., 2007). We hypothesise that the diatom composition will differ significantly between different substrates, that the species composition on each substrate will respond to an environmental gradient and that differences between the substrates will not affect the stream health assessment.

Materials and methods

Study area

Samples were taken from sites across the eastern and western Mount Lofty Ranges (EMLR and WMLR,

respectively), South Australia (Fig. 1) in the Austral spring of 2015 (see Table S1, in Supporting Information). The Mount Lofty Ranges (MLR) extend north from the southern tip of the Fleurieu Peninsula for 300 km and reach up to 936 m in altitude. The MLR are a watershed for regionally important water supply rivers such as the River Torrens, Onkaparinga River and North Para River, and smaller streams such as First, Brownhill and Deep Creeks, and divides the city of Adelaide from the Murraylands in the east. The MLR receive between 100 and 300 mm of rain in the study season of spring (Bureau of Meteorology, 2016) and have multiple land uses including residential development, a broad range of agriculture and horticulture, timber plantations and national and conservation parks.

Thirty-one sites in the EMLR and WMLR were selected from a data base of previously sampled streams to provide a comprehensive spatial coverage and range of environmental gradients from the best quality streams to some of the most degraded streams in South Australia. This quality assessment was made using multiple-lines of evidence approach (see http://www.epa.sa.gov.au/reports_water/amlr_creeks-ecosystem-2013) that incorporated the biological condition gradient concept from Davies & Jackson (2006). DSIAR scores from our sample sites from a previous rope-based sampling (Autumn, 2015) ranged from 36 to 61, representing over two-thirds of the full range of DSIAR site scores from a large (> 1000 sample) study of south-east Australian streams (Chessman et al., 2007). This suggests that the study region provides a sufficient gradient to assess the influence of substrate on DSIAR scores.

Habitat and chemistry data

Eighteen habitat and water chemistry variables were measured at each site to assess the major chemical, physical and biological characteristics (Table 1). These variables were chosen as they are likely to influence the composition, distribution and abundance of diatoms growing within the sampled streams.

Physical and chemical measurements (dissolved oxygen, pH, electrical conductivity, specific conductance and water temperature) were taken using a Yellow Springs Instrument multimeter (model 556) placed in a representative section of the stream (ideally within flowing water if present, otherwise

Fig. 1 Location of sample sites in the Mount Lofty Ranges (sites are marked with circles). Inset is the location of the map region in Australia

from within the channel). A composite water sample was collected from each site into a bucket from 5 to 6 representative locations in a 100 m reach at each site using a 1–2-litre container; the container was rinsed in the stream water prior to collecting the representative water sample from the site. Water samples were collected from the water mixed in the bucket and stored on ice until receipt by the laboratory for later analysis of total phosphorus (TP), total nitrogen (TN),

oxidised nitrogen (NO_x), total Kjeldahl nitrogen (TKN) and reactive silica using standard methods (APHA, 2005).

Physical measurements (current speed, stream width) were taken from a representative non-flowing channel and, if present, flowing riffle habitat within each site by recording the time for a floating leaf to traverse 1 m and using a measuring staff to record the wetted width of the stream. Visual estimates of the

Table 1 Environmental variables used in this study

Chemical	Physical	Biological	Location
Conductivity	Water temperature	Percent of channel covered by macrophytes	Latitude
pH	Mean depth	Percent of channel covered by filamentous algae	Longitude
Dissolved oxygen	Minimum current speed		
Oxidised nitrogen (NO _x)	Maximum current speed		
Total nitrogen (TN)	Width of stream		
Total phosphorus (TP)	Shade Cover		
Total Kjeldahl nitrogen (TKN)			
Silica reactive			

area of channel covered in both macrophytes and filamentous algae were made using the following percentages: none, < 10%, 10–35%, 35–65%, 65–90% and > 90%. The total amount of shade covering the stream was estimated visually by considering the shade provided by the stream banks, riparian vegetation and surrounding landscape during daylight hours.

Diatom collection and processing

The diatom collection process was undertaken in two stages. In the first stage, frayed ropes were deployed in streams between 19th October and 2nd November, 2015. Areas of unusual conditions (e.g. areas of extreme shade and/or turbulence) were avoided. We used white polypropylene rope (6 mm in diameter). The end 5 cm of the rope was isolated using a cable tie and frayed to increase the surface area of the sample (see Fig. 2). Three rope substrates were deployed at each site by affixing the non-frayed end of the rope to an immovable object, such as a submerged rock or overhanging branch. Each rope was long enough for the 5-cm-long frayed end to remain floating on the stream surface even when the water level changed, thus providing a uniform relationship to water depth (Tibby et al., 2019). The rope was left for between 6 and 8 weeks to allow diatom colonisation.

The second stage of sampling took place between the 19th November and 4th December 2015. Here, the ropes were retrieved and the frayed 5 cm end of each rope was collected for analysis. In addition, samples from rock and mud were also collected. Mud samples were collected by scraping 5 cm³ of sediment from the top of the river bed from within a 10 cm² area directly into a sampling tube. Rock samples were collected

Fig. 2 Rope substrate (white polypropylene) with 5-cm frayed end to enable diatom colonisation

from five rocks which were too large to be transported in the stream's flow at the time of sampling. Each rock was rinsed in the stream to remove any loose debris which may have been transported from upstream and a 5 cm² area on the upper face of the rock was scraped off using a sharp knife. Methylated spirit was added to the samples immediately after collection to preserve collected diatoms (Fell et al., 2018). Diatoms were processed in the lab within 48 h.

At 17 sites samples from all substrates were successfully obtained. At a further five sites, two out of the three substrates were sampled. No samples were collected from a further eight sites as they were either dry or could not be accessed (Table S1). Diatoms were counted from all collected samples but the 13 sites with incomplete substrate sets were excluded from all analyses (see supplementary material).

In the laboratory, all diatom samples were heated in a 50-ml centrifuge tube in a water bath for 3 h at 60–80°C in 25% hydrogen peroxide to remove organic material and, for the rope samples, this was undertaken to dislodge diatoms from each 5-cm rope end. The samples were then washed three times in distilled water and where applicable the rope was removed from the sample after being rinsed with distilled water

(Tibby et al., 2019). A high (200 µl) and low (100 µl) concentration of each sample was placed on a 22 × 22 mm coverslip and then mounted on slides using Naphrax®. Approximately 300 valves were counted on each slide using a Nikon eclipse E600 microscope at × 1500 magnification with a Nikon Plan Fluor 100 × 1.30 oil DIC H objective. Diatoms were identified to species level using Krammer & Lange-Bertalot (1986, 1988, 1991a, b) and Sonneman et al. (1999).

Statistical analyses

Diatom composition was charted in C2, a program that produces visualisations of paleoenvironmental data (Juggins, 2007). The five most abundant diatom species from each substrate were included. Differences in diatom diversity between substrates were evaluated using simple species richness and the Shannon Index (Shannon & Weaver, 1949). Species richness was used to emphasise the importance of rare species and the Shannon Index was used to account for both the number and evenness of species present in the samples. Only sample sites where all three substrates were collected were used for comparison.

The Diatom Species Index for Australian Rivers (DSIAR) (Chessman et al., 2007) was used to assess the effect of different substrates selection on stream health assessment. The DSIAR is sensitive to overall catchment disturbance, along with modelled nutrient and, to a lesser extent, suspended sediment loads (Chessman et al., 2007). It has been applied in a wide range of settings in Australia (e.g. Chessman & Townsend, 2010; Oeding & Taffs, 2017) and has also been shown to exhibit significant relationships to water quality in New Zealand wetlands (Kilroy et al., 2017). The DSIAR was developed and tested in a large study area that overlapped the present one (Chessman et al., 2007).

The index values were calculated using the following equation:

$$\text{Index Value} = \frac{\sum \text{SI} \times X_p}{\sum X_p}$$

where SI is the sensitivity value and X_p is the proportion each diatom species made up of the whole sample. The value is divided by the total proportion of diatoms with attributed SI numbers, to account for

some diatom species not having SI numbers in the DSIAR index. The DSIAR index has a possible range between 1 and 100. High index scores signify that the stream/river condition is comparatively natural. Low scores suggest the presence of anthropogenic stress on the stream/river.

Detrended correspondence analysis (DCA) was undertaken to assess the difference in diatom composition from different sites and substrates. The distance between samples ($n = 51$) plotted in ordination space represents the (dis)similarity between the samples. DCA is an unconstrained ordination technique appropriate to relative abundance data which have long gradient lengths; our data set had a gradient length > 3 SD units and therefore DCA is appropriate (Birks, 2010). An initial correspondence analysis exhibited an arch effect and so DCA was utilised. DCA was undertaken on square root transformed diatom relative abundances that exceeded 1% in at least one sample, in the vegan package (Oksanen et al., 2014) in R (R Core Team, 2018). Downweighting of rare species was not applied.

Canonical correspondence analysis (CCA) was used to assess the relationship between the diatom composition and environmental variables. CCA is a constrained ordination technique which identifies the environmental variables that have the greatest explanatory power in separating sites based on diatom compositional data. The CCAs were run with CANOCO 4.5 (Ter Braak, 1995) using Hill's scaling (Hill, 1973). Scaling focused on inter-sample distances and the forward selection of environmental variables used a Monte Carlo Permutation Test with 9999 permutations. Environment variables with $P < 0.05$ were considered significant. CCAs were run with square root transformed species data and log-transformed environment data.

Partial CCAs (pCCA) were also run with CANOCO 4.5 (Ter Braak, 1995) to assess the interrelationship between environmental variables. pCCAs allow the significance of an environmental variable to be tested when the influence associated with a different environmental variable (termed a co-variable) has been removed. In the pCCA, the co-variables (conductivity and TP) were chosen because they were significant in at least one of the data sets (rope, mud, rock). In both CCA and pCCA, we defined outliers as plotting at least four standard deviation units away from the mean. One rope sample from Mount Barker Creek (EMLR13)

fulfilled this criterion and was made passive. This site had the largest pool in our data set (> 4 m wide and > 20 m long) and was dominated by the planktonic diatom *Pantocsekiella comensis* (Grunow) K.T.Kiss & E.Ács.

Results

Diatom composition and diversity

208 diatom taxa were recorded (with 144, 94 and 94 taxa on rope, rock and mud, respectively) with *Rhoicosphenia abbreviata* (C.Agardh) Lange-Bertalot as the dominant species for all substrates, accounting for 14.1% of all values counted (Table 2). *Achnanthyidium minutissimum* (Kützing) Czarnecki and *Cocconeis placentula* Ehrenberg were also dominant across all substrates, comprising 9.5% and 7.0% of all valves counted, respectively. A number of other taxa were also highly abundant in some samples including *Nitzschia frustulum* (Kützing) Grunow, *Planothyidium delicatulum* (Kützing) Round & Bukhtiyarova and *Tabularia fasciculata* (C. Agardh) D.M.Williams & Round (Fig. 3). In addition, *Pseudostaurosira elliptica* (Schumann) Edlund, Morales & Spaulding was the second most dominant species in mud samples but was absent from the rope samples (Table 2, Fig. 3).

Some of the dominant diatoms were present along the full conductivity gradient but *Amphora pediculus* (Kützing) Grunow and *A. minutissimum* were only present above trace values ($> 1\%$) in streams with a conductivity below $2000 \mu\text{S cm}^{-1}$, while *T.*

fasciculata became increasingly dominant in streams with a conductivity above $3500 \mu\text{S cm}^{-1}$ (Fig. 3).

The mean diversity of diatom samples was lowest from the rope substrate and highest from the mud substrate and the greatest variance was seen on the rock substrate (Fig. 4). There was a significant difference between the species richness and the Shannon diversity index for each substrate (ANOVA, $P < 0.005$). The Detrended Correspondence Analysis indicated that the difference between the rock and the mud sample assemblage was, on average, smaller than the difference between the artificial and the natural substrates (Fig. 5). Diatom assemblages from rope tended to plot low on axis 1 and, in particular, high on axis 2. In comparison, the diatom assemblages from natural substrates were found high on axis 1 and lower on axis 2 (Fig. 5.). For a number of sites (e.g. EMLR03, WMLR08, WMLR15), the diatom assemblages from mud and rock were virtually indistinguishable in the DCA. In only a very small number of sites (e.g. WMLR11) were samples from all three substrates proximally located in the DCA ordination.

Diatom water quality relationships

When CCAs were individually performed for the rope, mud and rock diatom assemblages, conductivity accounted for 9.6%, 9.5% and 7.8% of variance, respectively (Table 3). This explained variance was significant in the rope and mud data sets, but not the rock data set. Indeed, the pCCA showed that conductivity was the environmental variable which explained the greatest amount of (significant) variance, independent of TP, in both the rope and mud data sets. TP did not explain any significant variance in the mud

Table 2 The most abundant diatom species for each substrate

	Rope		Rock		Mud	
	Species	Percentage (%)	Species	Percentage (%)	Species	Percentage (%)
1st	<i>Rhoicosphenia abbreviata</i>	17.9	<i>Rhoicosphenia abbreviata</i>	14.0	<i>Rhoicosphenia abbreviata</i>	10.5
2nd	<i>Cocconeis placentula</i>	16.6	<i>Achnanthyidium minutissimum</i>	11.3	<i>Pseudostaurosira elliptica</i>	8.7
3rd	<i>Achnanthyidium minutissimum</i>	10.5	<i>Amphora pediculus</i>	9.5	<i>Achnanthyidium minutissimum</i>	6.5

Fig. 3 The eight most abundant species ordered by increasing conductivity. AS = artificial substrate (i.e. rope), R = rock and M = mud

Fig. 4 The mean and one standard deviation for diatom **a** species richness and **b** Shannon Index on rope, rock and mud substrates

Fig. 5 Correspondence analysis of diatom compositions from the Mount Lofty Ranges. Samples taken from the same site are joined with a line. Replicate samples from the local study at site WMLR09 are depicted with smaller symbols

data set when conductivity was included as a co-variable (Table 5). Indeed, this situation was also true when TP was selected as the sole explanatory variable (6.7% explained; $P = 0.2409$).

For the rock substrate, CCA showed only total phosphorus was significant in explaining diatom variance (Table 3). The pCCA also showed that TP was the most important explanatory variable in the rock data set where it explained a significant amount of the variation independent of conductivity (as it did in the rope data set). No other variables, including those related to flow and shade (Table 1) which are common

drivers of diatom stream community structure (Hill, 1973; Hart & Finelli, 1999; Stevenson et al., 2010), explained significant variance in Mount Lofty Ranges diatom assemblages.

The DSIAR index values between rope, rock and mud were strongly correlated ($P < 0.005$; Table 4). For both rope and mud, WMLR07 was calculated to have the lowest and WMLR01 to have the highest DSIAR index score (Table 5). For rock, these sites had the 4th lowest and 2nd highest scores, respectively. The median values ranged from 40.80 (rope) to 43.28 (mud) with rope having the largest interquartile range and mud having the smallest, with ranges of 9.08 and 3.02, respectively (Table 5).

Discussion

Diatoms are widely used to investigate stream water quality, however, a standard methodology is lacking—especially from streams with variable water levels—resulting in a variety of natural and artificial substrates being used to collect diatoms (Kelly et al., 1998; Lane et al., 2003; Townsend & Gell, 2005). Rope has been identified as potentially being an optimal substrate since it eliminates many of the problems associated with natural substrates, such as a lack of consistent availability of a particular substrate or variation within substrate type (Kelly et al., 1998; Goldsmith, 2000; Tibby et al., 2019). However, the efficacy of rope has not been tested.

Table 3 The significance of the dominant environmental variable (as determined in the CCA) when checked for independence using individual partial Canonical Correspondence Analyses for each substrate

	Variables	Co-variables	Significance	Percentage explained
Rope	Conductivity	n/a	0.0069	9.6%
	Conductivity	TP	0.0145	9.6%
	TP	Conductivity	0.0430	8.3%
Rock	Conductivity	n/a	0.1361	7.8%
	TP	n/a	0.0338	8.6%
	TP	Conductivity	0.0458	8.4%
	Conductivity	TP	0.1157	7.6%
Mud	Conductivity	n/a	0.0121	9.5%
	Conductivity	TP	0.0685	8.2%
	TP	Conductivity	0.3764	5.3%

Significant variables are shown in bold. The variables used in the analysis are listed in Table 1

Table 4 Correlation matrix of DSIAR index values from rope, rock and mud substrates

	Rope	Rock	Mud
Rope		<0.005	<0.005
Rock	0.74		<0.005
Mud	0.91	0.81	

Only DSIAR index values from sites where samples from all three substrates were collected were included ($n = 17$)

Diatom composition and diversity

This study found each substrate tended to have a different diatom assemblage (Figs. 3 and 5), suggesting that the microhabitat of the substrate influences the species of diatoms present (Round, 1991). The difference between substrates could be caused by differences in the availability of nutrients (Stockner & Shortreed, 1978; Chételat et al., 1999; Stelzer & Lamberti, 2001), competition and/or the time diatoms had to colonise the substrates (Cattaneo & Amireault, 1992; Hillebrand & Sommer, 2000; Passy, 2007; B-Béres et al., 2014, 2016). For example, in some mud samples, *Pseudostaurosira elliptica* was dominant but was much less abundant on rock (6.77%). *Fragilaria* species (*sensu lato*) have previously been identified as having a strong association with mud substrates (Winter & Duthie, 2000). Interestingly, the composition on the ropes could be seen as reflecting diatom composition typically observed on plants. *Cocconeis placentula* was found over trace values ($> 1\%$) at twelve sites and in eleven of those it was dominant in the rope samples. *C. placentula* has previously been identified as a dominant species on plants due to its attachment by valve face and mucilage features (Soininen & Eloranta, 2004). Hence, it may be that the rope acts as a similar substrate to plants. In this

study it is possible that the rope, which floats at the stream surface, experienced higher velocities than the other sites within the reach, in particular those locations where mud settled. Hence the rope setting may suit low-profile diatoms such as *C. placentula* (Passy, 2007) which also have strong attachment mechanism (Lukács et al., 2018). By contrast to the overall pattern of taxon preferences for certain substrates, some taxa, including *Rhoicosphenia abbreviata* and, to a lesser extent, *Achnanthyidium minutissimum* exhibited relatively little preference for particular habitats (Fig. 3). In part, this may be due to the fact that these taxa are early colonisers of new or recently sloughed substrates (Passy, 2007; B-Béres et al., 2016).

The difference in species diversity on each substrate (Fig. 4) is likely to reflect different colonisation times for different substrates. In this context, it is recommended to leave ropes submerged in streams for at least four weeks to reduce the influence of early colonisers (Kelly et al., 1998). In this study, ropes were deployed for 6–8 weeks, and yet early coloniser species such as *C. placentula* and *A. minutissimum* (Patrick, 1977; Passy, 2007; Lukács et al., 2018) were often dominant (Fig. 3). Hence, the low levels of diversity on the rope are likely to have been influenced by colonisation time, a key driver of diversity in streams (B-Béres et al., 2016; Lukács et al., 2018). This raises the possibility that species diversity on the artificial substrates could have increased if they had been submerged for longer. This requires further research, however, since the potential advantages of increased submergence times need to be balanced against the requirement to undertake multiple water quality samples to characterise water quality over the deployment period. Furthermore, problems can emerge if the ropes are submerged during high flow events (preventing retrieval) or exposed (in ephemeral

Table 5 The minimum, 25% percentile, median, 75% percentile and maximum DSIAR index values for each substrate across all sites where substrates were successfully collected

	Minimum (site)	25% percentile	Median	75% percentile	Maximum (site)
Rope ($n = 17$)	34.91 (WMLR07)	37.91	40.80	47.02	59.97 (WMLR01)
Rock ($n = 17$)	35.75 (EMLR03)	37.94	41.94	46.17	60.29 (WMLR06)
Mud ($n = 17$)	37.50 (WMLR07)	41.65	43.28	44.68	57.67 (WMLR01)

The minimum and maximum values also note the sites at which they were recorded

streams that run dry during summer months). In epipellic (mud) samples, the maturity of biofilms and lower disturbance contributes to higher diatom diversity (Passy, 2007). However, higher species diversity in mud assemblages may also reflect the accumulation of senesced diatoms present before the sample period (Wilson & Holmes, 1981). In contrast, rock samples were rinsed before sampling in our sampling, with the aim of removing dead diatoms, and the rope was only exposed for the six to eight weeks, limiting the likelihood of dead diatoms being incorporated in samples from these substrates.

The influence of substrate on the assessment of index values and water quality relationships

Despite clear differences in diatom composition in the different substrates in our sites, and the possible influence of different traits on diatom water quality responses (Passy, 2007; Berthon et al., 2011; B-Béres et al., 2016; Lukács et al., 2018), DSIAR index values were significantly correlated between rope, rock and mud (Table 4) and also had similar distributions (Table 5). Indeed, the artificial substrate (rope) was more similar in terms of its DSIAR score to the natural substrate mud, than mud was to rock, the other natural substrate. This suggests that diatom-based stream health indices are robust to the choice of substrate and therefore, for studies using this index, the additional cost of deploying ropes in such studies may be unwarranted. As the DSIAR index was developed using a combination of substrates including rock, submerged logs and mud (Chessman et al., 2007), this may contribute to its wide applicability for different stream types in Australia.

However, while the overall assessment of stream health (i.e. the DSIAR) was unaffected by substrate type, the response of diatoms to individual environmental gradients was substrate dependent and therefore, in contrast, suggests that rope substrates are well suited to studies of nutrient status and sensitivity in streams. In particular, diatoms from rope and rock substrates exhibited a significant relationship to the concentration of total phosphorus (TP) in contrast to those from mud which did not. The lack of mud diatom response to stream water TP concentration is likely to arise from diatoms being able to utilise phosphorus in the mud, thereby reducing or eliminating physiological effects or competitive interactions in relation to

TP in the water (House 2003; Søndergaard et al., 2003). These effects may partly result from the different diatom guilds in the different habitats. Low-profile diatom taxa such as *Achnanthes minutissimum*, *Cocconeis placentula* and *Rhoicosphenia abbreviata*, which are earlier colonisers of substrates and well adapted to faster flowing waters (in our study likely to be the rope and rock substrates), are often the group most sensitive to changes in nutrients (Passy, 2007; Berthon et al., 2011). *Achnanthes minutissimum* and *Cocconeis placentula* also have strong attachment to substrates (Lukács et al., 2018). Conversely, motile taxa such as *Nitzschia frustulum* are more abundant in high nutrient biofilms where they can move to access additional nutrients (Passy, 2007), and secrete enzymes which allow them to trap nutrients (Berthon et al., 2011), and therefore may be less responsive to changes in nutrient concentrations in overlying waters (Passy, 2007; Berthon et al., 2011).

The absence of a conductivity response in the rock substrate is less readily explained. It seems unlikely that the lack of response of the epilithic (rock) diatom community to water quality can be attributed to the underlying influence of rock chemistry. Bergey (2008) has shown that rock chemistry is unlikely to influence periphyton (including diatom) composition in streams. Moreover, while differential responses of rock and mud diatom communities to water quality have been attributed to flow-related drivers (with rock diatom communities more adhesive and less vulnerable to sloughing) (Soininen, 2005; Bojorge-García et al., 2014), the lack of any significant response to flow in our study (see Table 4) makes such a response less likely. This result remains unexplained.

Conclusion

To assess the efficacy of rope as a substrate in diatom-based stream water quality assessments, samples from artificial substrates (rope), rock and mud were analysed from 17 sites. Substrate type did not affect the overall assessment of stream health based on the Diatom Species Index for Australian Rivers; however, the substrate type did appear to influence diatom–nutrient relationships. In particular, diatom samples from rock and rope substrates exhibited a significant relationship with total phosphorus while diatom

samples from mud did not. This is likely due to the mud substrate providing a source of nutrients unavailable to diatoms growing on rope and rock. Therefore, for diatom-based assessments of stream nutrient status, rope appears to be the most suitable sampling substrate since it has no available nutrients that can contribute to diatom growth (Goldsmith, 2000). Furthermore, rope is advantageous for reasons of its uniformity (which contrasts to the micro-scale variability of other artificial substrates such as bricks and tiles) and has a set deployment time. In addition, ropes could be deployed for longer periods of time than in our study to increase species diversity in the samples. Despite their advantages, the use of artificial substrate essentially doubles the field work-related cost of any monitoring program as it requires both deployment and retrieval. Hence, while there is clearly some benefit in the detection of nutrient response from rope substrates, it may be that the additional cost is not always worthwhile. In this context, if other variables (such as conductivity) are the focus and, or, if time or cost are limited, then natural substrates, in particular rocks and other hard surfaces, are likely to provide an adequate alternative. Further studies are required to investigate if the specific properties (material, colour, type etc.) of the rope used for sampling significantly impact the diatom composition.

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References

- Allan, I., B. Vrana, R. Greenwood, G. Mills, B. Roig & C. Gonzalez, 2006. A “toolbox” for biological and chemical monitoring requirements for the European Union’s Water Framework Directive. *Talanta* 69: 302–322.
- Bere, T. & J. G. Tundisi, 2011. The effects of substrate type on diatom-based multivariate water quality assessment in a tropical river (Monjolinho), São Carlos, SP, Brazil. *Water, Air, & Soil Pollution* 216: 391–409.
- B-Béres, V., P. Török, Z. Kókai, E. T. Krasznai, B. Tóthmész & I. Bácsi, 2014. Ecological diatom guilds are useful but not sensitive enough as indicators of extremely changing water regimes. *Hydrobiologia* 738: 191–204.
- B-Béres, V., Á. Lukács, P. Török, Z. Kókai, Z. Novák, E. Krasznai, B. Tóthmész & I. Bácsi, 2016. Combined ecomorphological functional groups are reliable indicators of colonisation processes of benthic diatom assemblages in a lowland stream. *Ecological Indicators* 64: 31–38.
- Berthon, V., A. Bouchez & F. Rimet, 2011. Using diatom life-forms and ecological guilds to assess organic pollution and trophic level in rivers: a case study of rivers in south-eastern France. *Hydrobiologia* 673: 259–271.
- Bergey, E. A., 2008. Does rock chemistry affect periphyton accrual in streams? *Hydrobiologia* 614: 141–150.
- Bojorge-García, M., J. Carmona & R. Ramírez, 2014. Species richness and diversity of benthic diatom communities in tropical mountain streams of Mexico. *Inland Waters* 4: 279–292.
- Botwe, P. K., L. A. Barmuta, R. Magierowski, P. McEvoy, P. Goonan & S. Carver, 2015. Temporal patterns and environmental correlates of macroinvertebrate communities in temporary streams. *PLoS ONE* 10: e0142370.
- Boulton, A. J., 1999. An overview of river health assessment: philosophies, practice, problems and prognosis. *Freshwater Biology* 41: 469–479.
- Bunn, S. E., E. G. Abal, M. J. Smith, S. C. Choy, C. S. Fellows, B. D. Harch, M. J. Kennard & F. Sheldon, 2010. Integration of science and monitoring of river ecosystem health to guide investments in catchment protection and rehabilitation. *Freshwater Biology* 55: 223–240.
- Bureau of Meteorology, 2016. Climate data online. www.bom.gov.au/climate/data.
- Cantonati, M. & D. Spitale, 2009. The role of environmental variables in structuring epiphytic and epilithic diatom assemblages in springs and streams of the Dolomiti Bellunesi National Park (south-eastern Alps). *Fundamental and Applied Limnology/Archiv für Hydrobiologie* 174: 117–133.
- Cattaneo, A. & M. C. Amireault, 1992. How artificial are artificial substrata for periphyton? *Journal of the North American Benthological Society* 11: 244–256.
- Chessman, B. C., N. Bate, P. A. Gell & P. Newall, 2007. A diatom species index for bioassessment of Australian rivers. *Marine and Freshwater Research* 58: 542.
- Chessman, B. C. & S. A. Townsend, 2010. Differing effects of catchment land use on water chemistry explain contrasting behaviour of a diatom index in tropical northern and temperate southern Australia. *Ecological Indicators* 10: 620–626.

- Chételat, J., F. R. Pick, A. Morin & P. B. Hamilton, 1999. Periphyton biomass and community composition in rivers of different nutrient status. *Canadian Journal of Fisheries and Aquatic Sciences* 56: 560–569.
- Dalu, T., A. W. E. Galloway, N. B. Richoux & P. W. Froneman, 2016. Effects of substrate on essential fatty acids produced by phytobenthos in an austral temperate river system. *Freshwater Science* 35: 1189–1201.
- Dalu, T., N. B. Richoux & P. W. Froneman, 2014. Using multivariate analysis and stable isotopes to assess the effects of substrate type on phytobenthos communities. *Inland Waters* 4: 397–412.
- Davies, S. P. & S. K. Jackson, 2006. The biological condition gradient: a descriptive model for interpreting change in aquatic ecosystems. *Ecological Applications: A Publication of the Ecological Society of America* 16: 1251–1266.
- Fell, S. C., J. L. Carrivick, M. G. Kelly, L. Füreder & L. E. Brown, 2018. Declining glacier cover threatens the biodiversity of alpine river diatom assemblages. *Global Change Biology* 24: 5828–5840.
- Gabel, K. W., J. D. Wehr & K. M. Truhn, 2012. Assessment of the effectiveness of best management practices for streams draining agricultural landscapes using diatoms and macroinvertebrates. *Hydrobiologia* 680: 247–264.
- Goldsmith, B. J., 1996. A rationale for the use of artificial substrata to enhance diatom-based monitoring of eutrophication in lowland rivers. Research paper No. 13, Environmental Change Research Centre, University College, London.
- Goldsmith, B. J., 2000. A diatom-based model to monitor trophic status in lowland rivers using artificial substrata. University College, London.
- Hering, D., R. K. Johnson, S. Kramm, S. Schmutz, K. Szoszkiewicz & P. F. M. Verdonshot, 2006. Assessment of European streams with diatoms, macrophytes, macroinvertebrates and fish: a comparative metric-based analysis of organism response to stress. *Freshwater Biology* 51: 1757–1785.
- Hill, M. O., 1973. Diversity and evenness: a unifying notation and its consequences. *Ecology* 54: 427–432.
- Hillebrand, H. & U. Sommer, 2000. Diversity of benthic microalgae in response to colonization time and eutrophication. *Aquatic Botany* 67: 221–236.
- House, W. A., 2003. Geochemical cycling of phosphorus in rivers. *Applied Geochemistry* 18: 739–748.
- Juggins, S., 2007. C2, Software for Ecological and Palaeoecological Data Analysis and Visualisation. Newcastle University, Newcastle Upon Tyne.
- Jüttner, I., H. Rothfritz & S. J. Ormerod, 1996. Diatoms as indicators of river quality in the Nepalese Middle Hills with consideration of the effects of habitat-specific sampling. *Freshwater Biology* 36: 475–486.
- Karr, J. R., 1999. Defining and measuring river health. *Freshwater Biology* 41: 221–234.
- Karthick, B., J. C. Taylor, M. K. Mahesh & I. V. Ramachandra, 2010. Protocols for collection, preservation and enumeration of diatoms from aquatic habitats for water quality monitoring in India. *The IUP Journal of Soil and Water Sciences* 3: 1–60.
- Kelly, M. G., A. Cazaubon, E. Coring, A. Dell’Uomo, L. Ector, B. Goldsmith, H. Guasch, J. Hürlimann, A. Jarlman, B. Kawecka, J. Kwandrans, R. Laugaste, E.-A. Lindström, M. Leitao, P. Marvan, J. Padisák, E. Pipp, J. Prygiel, E. Rott, S. Sabater, H. van Dam & J. Vizinet, 1998. Recommendations for the routine sampling of diatoms for water quality assessments in Europe. *Journal of Applied Phycology* 10: 215–224.
- Kilroy, C., A. M. Suren, J. A. Wech, P. Lambert & B. K. Sorrell, 2017. Epiphytic diatoms as indicators of ecological condition in New Zealand’s lowland wetlands. *New Zealand Journal of Marine and Freshwater Research* 51: 505–527.
- Krammer, K., & H. Lange-Bertalot, 1986. *Bacillariophyceae*. 1. Teil: Naviculaceae. Gustav Fischer, Jena.
- Krammer, K., & H. Lange-Bertalot, 1988. *Bacillariophyceae*. 2. Teil: Bacillariaceae, Epithemiaceae, Surirellaceae. Jena.
- Krammer, K., & H. Lange-Bertalot, 1991a. *Bacillariophyceae*. 3. Teil: Centrales, Fragilariaceae, Eunotiaceae. Gustav Fischer, Stuttgart.
- Krammer, K., & H. Lange-Bertalot, 1991b. *Bacillariophyceae*. 4: Achnanthes, Kritische Ergänzung zu Navicula (Lineolatae) und Gomphonema Gesamtliteraturverzeichnis Teil 1–4. Gustav Fischer, Stuttgart.
- Lane, C. M., K. H. Taffs & J. L. Corfield, 2003. A comparison of diatom community structure on natural and artificial substrata. *Hydrobiologia* 493: 65–79.
- Lukács, Á., Z. Kókai, P. Török, I. Bácsi, G. Borics, G. Várbíró, et al., 2018. Colonisation processes in benthic algal communities are well reflected by functional groups. *Hydrobiologia* 823: 231–245.
- Madrid, Y. & Z. P. Zayas, 2007. Water sampling: traditional methods and new approaches in water sampling strategy. *TrAC Trends in Analytical Chemistry Elsevier* 26: 293–299.
- Mangadze, T., T. Dalu & P. William Froneman, 2019. Biological monitoring in southern Africa: a review of the current status, challenges and future prospects. *Science of the Total Environment* 648: 1492–1499.
- Ndaruga, A. M., G. G. Ndiritu, N. N. Gichuki & W. N. Wamicha, 2004. Impact of water quality on macroinvertebrate assemblages along a tropical stream in Kenya. *African Journal of Ecology* 42: 208–216.
- Newall, P., N. Bate & L. Metzeling, 2006. A comparison of diatom and macroinvertebrate classification of sites in the Kiewa River system, Australia. *Hydrobiologia* 572: 131–149.
- Norris, R. H. & K. R. Morris, 1995. The need for biological assessment of water quality: Australian perspective. *Austral Ecology* 20: 1–6.
- Oeding, S. & K. H. Taffs, 2015. Are diatoms a reliable and valuable bio-indicator to assess sub-tropical river ecosystem health? *Hydrobiologia* 758: 151–169.
- Oeding, S. & K. H. Taffs, 2017. Developing a regional diatom index for assessment and monitoring of freshwater streams in sub-tropical Australia. *Ecological Indicators* 80: 135–146.
- Oksanen, J., F. G. Blanchet, M. Friendly, R. Kindt, P. Legendre, D. McGinn, P. R. Minchin, R. B. O’Hara, G. L. Simpson, P. Solymos, M. H. M. Stevens, E. Szoecs, & H. Wagner, 2014. *Vegan: Community Ecology Package*. R Package Version 2.2-0. <http://CRAN.Rproject.org/package=vegan>.

- Passy, S. I., 2007. Diatom ecological guilds display distinct and predictable behavior along nutrient and disturbance gradients in running waters. *Aquatic Botany* 86: 171–178.
- Patrick, R., 1977. Ecology of freshwater diatoms and diatom communities. In Werner, D. (ed.), *The Biology of Diatoms*. University of California Press, Berkley: 284–332.
- Philibert, A., P. Gell, P. Newall, B. Chessman & N. Bate, 2006. Development of diatom-based tools for assessing stream water quality in south-eastern Australia: assessment of environmental transfer functions. *Hydrobiologia* 572: 103–114.
- Potapova, M. & D. F. Charles, 2005. Choice of substrate in algae-based water-quality assessment. *Journal of the North American Benthological Society* 24: 415–427.
- Potapova, M. G. & D. F. Charles, 2002. Benthic diatoms in USA rivers: distributions along spatial and environmental gradients. *Journal of Biogeography* 29: 167–187.
- R Core Team, 2018. R: A Language and Environment for Statistical Computing. Foundation for Statistical Computing, Vienna.
- Resh, V. H., 2008. Which group is best? Attributes of different biological assemblages used in freshwater biomonitoring programs. *Environmental Monitoring and Assessment* 138: 131–138.
- Round, F. E., 1991. Diatoms in river water-monitoring studies. *Journal of Applied Phycology* 3: 129–145.
- Schofield, N. J. & P. E. Davies, 1996. Measuring the health of our rivers. *Water* 23: 39–43.
- Shannon, C. E. & W. Weaver, 1949. *The Mathematical Theory of Communication*. The University of Illinois Press, Illinois.
- Smucker, N. J. & M. L. Vis, 2010. Using diatoms to assess human impacts on streams benefits from multiple-habitat sampling. *Hydrobiologia* 654: 93–109.
- Soininen, J., 2005. Assessing the current related heterogeneity and diversity patterns of benthic diatom communities in a turbid and clear water river. *Aquatic Ecology* 38: 495–501.
- Soininen, J. & P. Eloranta, 2004. Seasonal persistence and stability of diatom communities in rivers: are there habitat specific differences? *European Journal of Phycology* 39: 153–160.
- Søndergaard, M., J. P. Jensen & E. Jeppesen, 2003. Role of sediment and internal loading of phosphorus in shallow lakes. *Hydrobiologia* 506–509: 135–145.
- Sonneman, J. A., A. Sincok, J. Fluin, M. Reid, P. Newall & J. Tibby, 1999. An illustrated guide to the common stream diatom species from temperate Australia. Cooperative Research Centre for Freshwater Ecology, Thurgooona.
- Stelzer, R. S. & G. A. Lamberti, 2001. Effects of N:P ratio and total nutrient concentration on stream periphyton community structure, biomass, and elemental composition. *Limnology and Oceanography* 46: 356–367.
- Stevenson, J. R., Y. Pan & H. Van Dam, 2010. Assessing environmental conditions in rivers and streams with diatoms. In Smol, J. P., J. Stoermer & F. Eugene (eds.), *The diatoms: applications for the environmental and earth sciences*. Cambridge University Press, Cambridge: 11–40.
- Stevenson, R. J. & S. Hashim, 1989. Variation in diatom community structure among habitats in sands streams. *Journal of Phycology* 25: 678–686.
- Stockner, J. G. & K. R. S. Shortreed, 1978. Enhancement of autotrophic production by nutrient addition in Carnation Creek, a coastal rainforest stream on Vancouver Island. *Journal of the Fisheries Research Board of Canada*. <https://doi.org/10.1139/f78-004>.
- Tan, X., Q. Zhang, M. A. Burford, F. Sheldon & S. E. Bunn, 2017. Benthic diatom based indices for water quality assessment in two subtropical streams. *Frontiers in Microbiology* 8: 601.
- Ter Braak, C. J. F., 1995. Ordination. In Jongman, R. H., C. J. Ter Braak & O. F. Van Tongeren (eds.), *Data analysis in community and landscape ecology*. Cambridge University Press, Cambridge: 91–173.
- Tibby, J., J. Richards, J. J. Tyler, C. Barr, J. Fluin & P. Goonan, 2019. Diatom-water quality thresholds in South Australia in streams indicate the need for more stringent water quality guidelines. *Marine and Freshwater Research*. <https://doi.org/10.1071/MF19065>.
- Townsend, S. A. & P. A. Gell, 2005. The role of substrate type on benthic diatom assemblages in the daly and roper rivers of the australian wet/dry tropics. *Hydrobiologia* 548: 101–115.
- Tsoi, W. Y., W. L. Hadwen & F. Sheldon, 2017. How do abiotic environmental variables shape benthic diatom assemblages in subtropical streams? *Marine and Freshwater Research* 68: 863–877.
- Tuchman, M. L. & R. J. Stevenson, 1980. Comparison of clay tile, sterilized rock, and natural substrate diatom communities in a small stream in Southeastern Michigan, USA. *Hydrobiologia* 75: 73–79.
- Wilson, C. J. & R. W. Holmes, 1981. The ecological importance of distinguishing between living and dead diatoms in estuarine sediments. *British Phycological Journal* 16: 345–349.
- Winter, J. G. & H. C. Duthie, 2000. Stream epilithic, epipelic and epiphytic diatoms: habitat fidelity and use in biomonitoring. *Aquatic Ecology* 34: 345–353.
- Wojtal, A. Z. & Ł. Sobczyk, 2012. The influence of substrates and physicochemical factors on the composition of diatom assemblages in karst springs and their applicability in water-quality assessment. *Hydrobiologia* 695: 97–108.
- World Health Organisation, 2011. *Guidelines for Drinking-water Quality*. WHO, Geneva.

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