

Are diatoms a reliable and valuable bio-indicator to assess sub-tropical river ecosystem health?

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Abstract The use of diatoms as bio-indicators of water quality is common in temperate regions worldwide. However, less attention has been accorded to sub-tropical regions, particularly in Australia. This study assessed the value of diatom communities to infer water quality in a sub-tropical riverine system. Epilithic diatom and water samples were collected monthly from an east Australian river. Principal components analysis showed that the Lower Catchment sites were more strongly influenced by the measured environmental variables differentiating them from the Upper Catchment sites. Canonical Correspondence Analysis showed electrical conductivity and total phosphorus strongly influenced the diatom community distribution. The study revealed diatom species that are robust bio-indicators of water quality in this sub-tropical catchment. *Cocconeis placentula*, *C. placentula* var *lineata*, *Gomphonema spec 2* and *Tabellaria flocculosa* were identified as indicators of moderate water quality. *Bacillaria*

paradoxa, *Navicula cryptocephala*, *Navicula mutica* var *mutica* and *Achnanthes fagedii* were identified as indicators of poor water quality. This study identified that diatoms are effective indicators of water quality. Further research is required to develop a diatom biological index applicable to sub-tropical east Australian river systems to improve the effectiveness of environmental monitoring and sustainable river management.

Keywords Australia · Diatom index · Bio-indicator · River management · Water quality

Introduction

Growing human demands on finite water resources has placed a burden on global riverine ecosystems (Wetzel, 2001; Dodson, 2005; Dodds & Whiles, 2010). As world population continues to grow exponentially, demand from multiple anthropogenic uses and ensuing degradation of water resources, is accelerating at an alarming rate (Wetzel, 2001; Jewitt, 2002; Dodson, 2005; Verhoeven & Setter, 2010). Freshwater ecosystems have seen declines in biodiversity far greater than any terrestrial ecosystem globally and are increasingly vulnerable to anthropogenic influence and environmental change (Dudgeon et al., 2006). Broad scale land use change alters hydrological, chemical and biological cycles resulting in catchment modification and degradation (Davies & Nelson, 1994; Hanson

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et al., 1994; Eyre, 1997; Peters et al., 1997). Unfortunately the result is the declining health of riverine ecosystems with subsequent loss of water quality and biodiversity (Wetzel, 2001; Millennium Ecosystem Assessment, 2005; Verhoeven & Setter, 2010). It is therefore becoming increasingly important to manage the health and integrity of natural water resources effectively to ensure a continued fresh water supply for both human populations and natural systems (Bunn et al., 1999; Preston, 2009; Axelrod, 2011). This has led to an increased focus on catchment scale research and the development of bio-indicators for assessment and monitoring programs to identify the health of riverine ecosystems.

The use of aquatic biota in environmental impact assessments and stream monitoring programs has gained momentum worldwide over the last few decades (Bellinger et al., 2006; Atazadeh et al., 2007). Bio-monitoring is a valuable management tool in assessment and achievement of water quality objectives and environmental values (Herricks & Schaeffer, 1985; ANZECC, 2000). It is integral to assess changes in riverine ecosystems whether they are due to changes in water quality, physical habitat or biological interactions (ANZECC, 2000). The use of bio-indicators gives a more thorough evaluation of environmental conditions. Physical and chemical analyses provide indications of water quality at the time of sampling while biotic parameters show environmental conditions over a temporal range (Battarbee et al., 2001; Philibert et al., 2006; Salomoni et al., 2006; Smol, 2008; Li et al., 2010; Lobo et al., 2010; Bere & Tundisi, 2011).

Benthic macro invertebrates have generally been the most commonly used group of organisms (Resh & Jackson, 1993; Norris & Norris, 1995; Atazadeh et al., 2007; Resh, 2008).

A move towards incorporating benthic diatoms into bio-monitoring programs particularly in Europe, North America and some parts of Australia has been occurring over the past few decades and are increasingly the preferred group of bio-indicators (Atazadeh et al., 2007; Kelly et al., 2009a; Smol & Stoermer, 2010; Almeida & Feio, 2012; Elias et al., 2012). Diatoms are a widely used, tested and proven bio-indicator. Diatoms have been used and researched extensively as bio-indicators of water quality, particularly in the temperate regions of the Northern Hemisphere (Kelly & Whitton, 1998; Wu & Kow,

2002; Bellinger et al., 2006; Salomoni et al., 2006; Bere & Tundisi, 2011). Over the last decade they have become an integral component of policy and legislation in environmental monitoring. In 2000, the Water Framework Directive (EC, 2000) was introduced in the European Union requiring the use of biological indicators (aquatic macrophytes, fishes, invertebrates and phyto-benthos) in the water quality evaluation, diatoms being the preferred indicator group by most European nations (Kelly et al., 2009a; Almeida & Feio, 2012; Elias et al., 2012). The United States Environmental Protection Agency systematically uses diatoms in their water quality assessments in many states (Smol & Stoermer, 2010).

Although considerable research effort has been given to temperate regions of the world, less attention has been paid to sub-tropical and tropical regions (Wu & Kow, 2002; Bellinger et al., 2006; Bere & Tundisi, 2011). Recently, a number of studies have been undertaken in temperate sub-tropical Brazil, developing methodology for the implementation of diatom-based monitoring programs in ecological status of riverine ecosystems (Salomoni et al., 2006, 2011; Lobo et al., 2010; Bere & Tundisi, 2011; Bohm et al., 2013). However, research in Australia has predominantly been focused in temperate zones (Blinn et al., 2004; Gell et al., 2005; Newall & Walsh, 2005; Newall et al., 2006; Haynes et al., 2011). Limited studies in sub-tropical regions have concentrated on paleolimnological studies (Parr et al., 2004; Taffs et al., 2008; Logan et al., 2010; Tibby & Taffs, 2011; Logan & Taffs, 2013), or on the estuarine environment (Townsend & Gell, 2005; Logan et al., 2010; Tibby & Taffs, 2011; Logan & Taffs, 2013), with comparatively negligible research effort on freshwater lotic systems (Lake, 1995; Blinn & Bailey, 2001; Mosisch et al., 2001).

Diatom indices have been well developed in the Northern Hemisphere, particularly in Europe and the US. However, the applicability of these indices to Australian rivers has not been widely researched. Diatoms have been under-utilised in Australia. Certainly, there are gaps in Australian diatom research, particularly in sub-tropical and tropical zones. The Diatom Index for Australian Rivers (DIARs) was developed by Chessman et al. (1999) as a tool (using 55 indicator genera) for assessing common anthropogenic stressors in eastern NSW and Victoria. This index assigned each genus with a number from 1 to 10 according to their inferred sensitivity to a general

range of stressors. This index was later improved upon by Chessman et al. (2007) using data from four Australian states and the Australian Capital Territory, extending the index to a species level version, the Diatom Species Index for Australian Rivers (DSIARs). This new index could potentially give a more accurate assessment of stream health, as it identifies species sensitivity variations within a genus, and negates previous problems with changes in diatom genera taxonomy (Chessman et al., 2007).

The data used to develop the DSIAR was sourced predominantly from temperate zones within Australia. This could limit its use in sub-tropical regions as it may not include enough datasets from sub-tropical and tropical zones for effective assessment in those areas. Lack of research in these climate zones may have limited the datasets available for inclusion in the DSIAR. These need to be developed to enhance the use of diatoms as bio-indicators, and implemented routinely and systematically as standard world's best practice in the assessment and monitoring of river health in Australia.

The aim of this study was to assess the diatom assemblages and water quality of a sub-tropical east Australian riverine system to identify a correlation between community composition and water chemistry and to assess the applicability of temperate region-based diatom indices to a sub-tropical river system.

Methods

Study area and sampling design

Coopers Creek (Fig. 1) is one of 23 sub-catchments of the Richmond River in northern NSW, with a total stream length of approximately 70 km (Singh et al., 2009). The sub-catchment is divided into two management zones. The Upper Coopers Creek Management Zone is characterised by steep forested slopes interspersed with small cleared rural properties, predominantly macadamia plantations. Much of the Upper Catchment is within NSW National Parks and Wildlife Service estates and has minimal anthropogenic influence. The Lower Coopers Creek Management Zone has extensively cleared valleys with little remaining remnant vegetation and wide floodplains with high productivity (Rous Water, 2009b). Land cleared for agriculture is dominated by beef and

dairy production and macadamia orchards, the predominant contributors to diffuse and point source pollution contributing to poor water quality (Morand, 1994; Aplin et al., 1999; Rous Water, 2009a; Singh et al., 2009). The riparian zones along the creeks are minimal and exotic weeds are a significant problem (Morand, 1994; Rous Water, 2009a).

Eight sample sites within the Coopers Creek sub-catchment (Fig. 1) were selected based on ease of access and appropriateness for diatom sampling, such as riffle zones with suitable substrata, depth/photic zone and light regime and were sampled monthly from March to August 2014. Sites 1–4 in the Upper Catchment had extensive riparian zones and were mostly within protected areas Sites 5–8 in the Lower Catchment were highly disturbed with minimal riparian zones, adjacent to macadamia orchards, nurseries and cattle grazing (dairy and beef).

At each site in situ physicochemical parameters were measured and samples were taken for nutrient and diatom analysis. A YSI 556 MPS multi meter was used onsite to determine water temperature, electrical conductivity (EC), pH and dissolved oxygen (DO). Water samples were collected in 1 L plastic bottles which were rinsed three times before sample collection at a depth of 200 mm. Samples were collected, kept on ice and transported back to the Southern Cross University Environmental Analysis Laboratory for further analysis [total dissolved salts, total suspended solids (TSS), phosphate (OP), nitrate (NO₃), nitrite (NO₂), ammonium (NH₃), total nitrogen (TN), total phosphorus (TP)] by a National Association of Testing Authorities accredited laboratory using standard APHA procedures (2012).

Epilithic diatom samples were obtained at each site following the method of Kelly et al. (1998). A sample of 1–3 ml was collected from a minimum of five rocks, randomly selected and sampled at a depth of approximately 200 mm. The upper surfaces of rocks were sampled by removing the algal growth with a toothbrush and collecting the sample in a labelled 70 ml plastic specimen container. All samples were kept on ice and transported back to the laboratory for further processing.

Laboratory analysis

Diatom samples were processed following the method of Battarbee et al. (2001). Soluble salts and

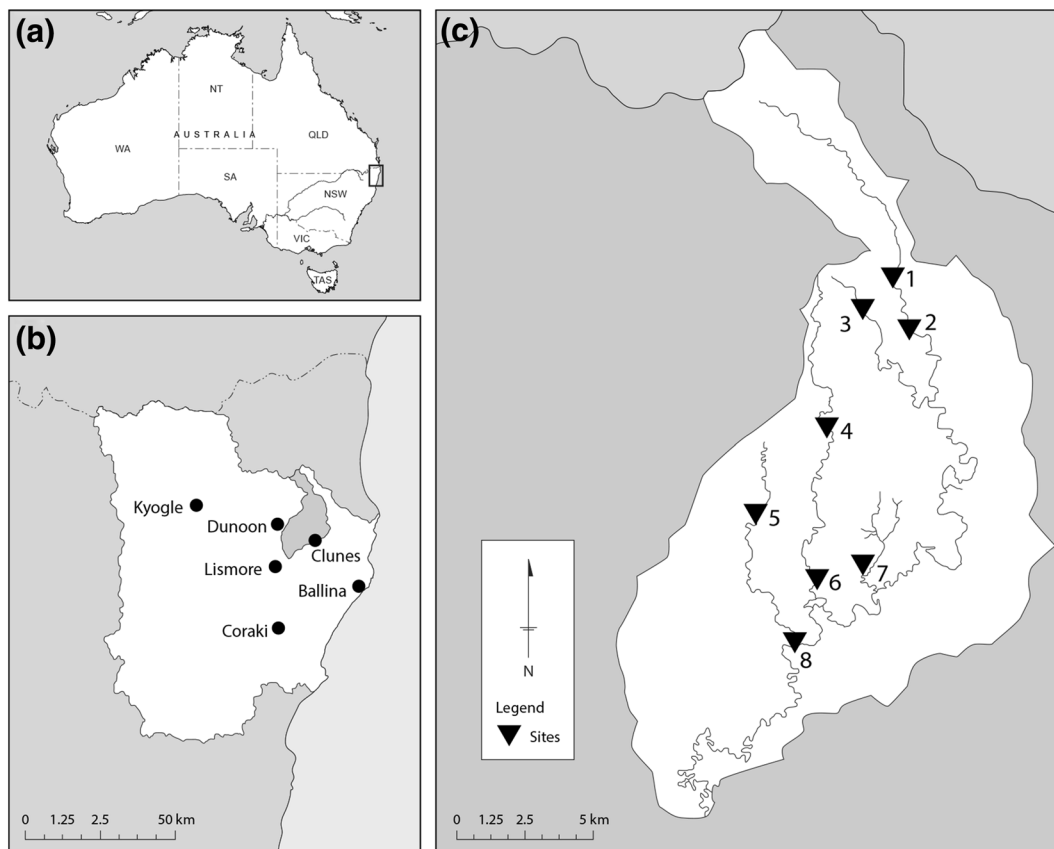


Fig. 1 Location of the Coopers Creek Catchment. **a** New South Wales, **b** Richmond River Catchment, **c** Coopers Creek Catchment with sample sites identified

carbonaceous material were removed with 10 % hydrochloric acid (HCl). Organic matter was removed by oxidation with 10 % hydrogen peroxide (H_2O_2). Two slides were prepared for each sample at a high and low density using the mounting medium Naphrax. A minimum of 300 diatom frustules (Battarbee et al., 2001; Chessman et al., 2007) were counted at $\times 1000$ magnification with oil emersion using an Olympus BX51 compound microscope. Diatoms were identified to species level wherever possible using information and photographic plates from a variety of publications including; Foged (1978), Krammer & Lange-Bertalot (1986, 1988, 1991a, b), Vyverman (1995), Hodgson et al. (1997) and Sonneman et al. (2000). All photos of diatom species are archived with the author.

Statistical analysis

Multivariate statistical analyses were used to identify major environmental gradients, explore diatom–

environment relationships and identify environmental variables that explained independent portions of the variance in the diatom data. Data were analysed using the statistical package R (R Development Core Team, 2006). Prior to statistical analysis, each environmental variable was checked for skewness, and EC, TP and TSS were $\log(x + 1)$ transformed. Principal components analysis (PCA) was performed on the environmental data to determine the major environmental gradients. Parametric *t* test analyses were performed to determine significant differences in environmental variables and diversity between Upper and Lower Catchment sites (level of significance $\alpha = 0.05$). Cluster analysis based on Euclidean distance was conducted on the water quality dataset. Detrended Correspondence Analysis with detrending by segments and down weighting of rare species was performed on the species data to establish whether species distribution was unimodal or linear. As gradient lengths were greater than two standard

deviation units, unimodal ordination techniques were used (Ter Braak, 1995). Species data were $\log(x + 1)$ transformed in an attempt to stabilise the variance in the dataset (Birks et al., 2001).

A series of Canonical Correspondence Analyses (CCA) were performed with scaling focused on inter-species distances, biplot scaling and down weighting of rare species. Variance inflation factors (VIFs) were identified and any environmental variables with VIFs >10 were removed. A series of CCAs of each environmental variable alone was performed, followed by CCAs of individual environmental variables with the remainder as co-variables (i.e., forward selection) to determine which made independent, significant contributions to explaining the variation in the species data (i.e., $P < 0.05$, based on 999 Monte Carlo permutation tests without Bonferroni or other adjustments). Variance partitioning was used to determine the amount of variation explained by each variable and the interactions between them.

The counts of each diatom taxon were expressed as a percentage of the total valves counted (relative abundance). Diatom community diversity was calculated for each sample using Shannon's diversity index (Cooper, 1995). Dominant diatom taxa $>5\%$ was included in the statistical analysis following Taffs et al. (2008), Bere & Tundisi (2009), Antón-Garrido et al. (2013) and O'Driscoll et al., (2014). Dominant taxa and species diversity was portrayed through stratigraphical diagrams using the program C2 (Juggins, 2007). The correlation between the dominant diatom species and the environmental parameters was calculated and those with an r^2 value >0.5 portrayed to explore species–environment relationships.

Results

Physicochemical data

Water quality analyses identified a trend of higher TP ($P = 5.55E-05$), TN ($P = 0.009$) and EC ($P = 8.87E-09$) values in the Lower Catchment, differentiating them from the Upper Catchment (Fig. 2; Table 1). TP values were high across all sites with the highest means in the Lower Catchment almost four times the ANZECC trigger value guideline (0.020 mg/l; ANZECC, 2000). Most sites recorded

TN mean values exceeding the ANZECC trigger value (0.250 mg/l; ANZECC, 2000). Upper Catchment sites recorded lower conductivity (mean 61 $\mu\text{S}/\text{cm}$) than Lower Catchment sites (mean 91.9 $\mu\text{S}/\text{cm}$). All sites recorded acidic values slightly lower than optimum pH. DO levels were below the ANZECC trigger value optimum of 90–110% saturation at all sites (highest 70.5 % sat) (see supplementary information for full dataset).

Analysis of environmental data

The PCA showed no clear dominant environmental parameter influencing the sites (Fig. 3). Temperature, EC, TP and pH are negatively correlated with the first PC axis. TN and DO are correlated with the second PC axis. The Upper Catchment sites predominantly fall in the positive PC axis 1 values. Site 2 had a strong correlation with DO. Site 8 had a strong correlation with temperature and TSS. Site 3 was notably different from all other sites, clustered in the upper right quadrant of the PCA. The Lower Catchment sites were more strongly influenced by the variables measured. The axis lengths were >2 ; therefore, a CCA analysis was conducted to explore species/environment relationships.

Cluster analysis of selected environmental variables (Fig. 4) indicated a distinction between the four sites of the Upper Coopers Creek (Sites 1–4) and the four sites of the Lower Coopers Creek Catchment (Sites 5–8). Sites separated at the first level are predominantly associated with those only of the Upper Catchment and sites predominantly associated with the Lower Catchment. At the next level, there are six groups: Group A—made up of three outliers of the Upper Catchment sites, Group B—mostly the Lower Catchment Sites 6–8, Group C—mostly Site 5, Group D—Sites 3 and 4 of the Upper Catchment, Group E—Sites 1, 2 and 4 and Group F—mostly Sites 1 and 2 with one of each Sites 3 and 4.

An initial CCA indicated that the environmental data explained 35.48 % of the variation in the diatom data (Table 2). As all VIFs were <10 , all environmental variables were retained. TP, pH and EC were correlated with axis 1 and DO, TSS, temperature and TN were correlated with axis 2 (Fig. 5). Six variables explained independent portions of the variance in the diatom data (as determined by forward selection) and

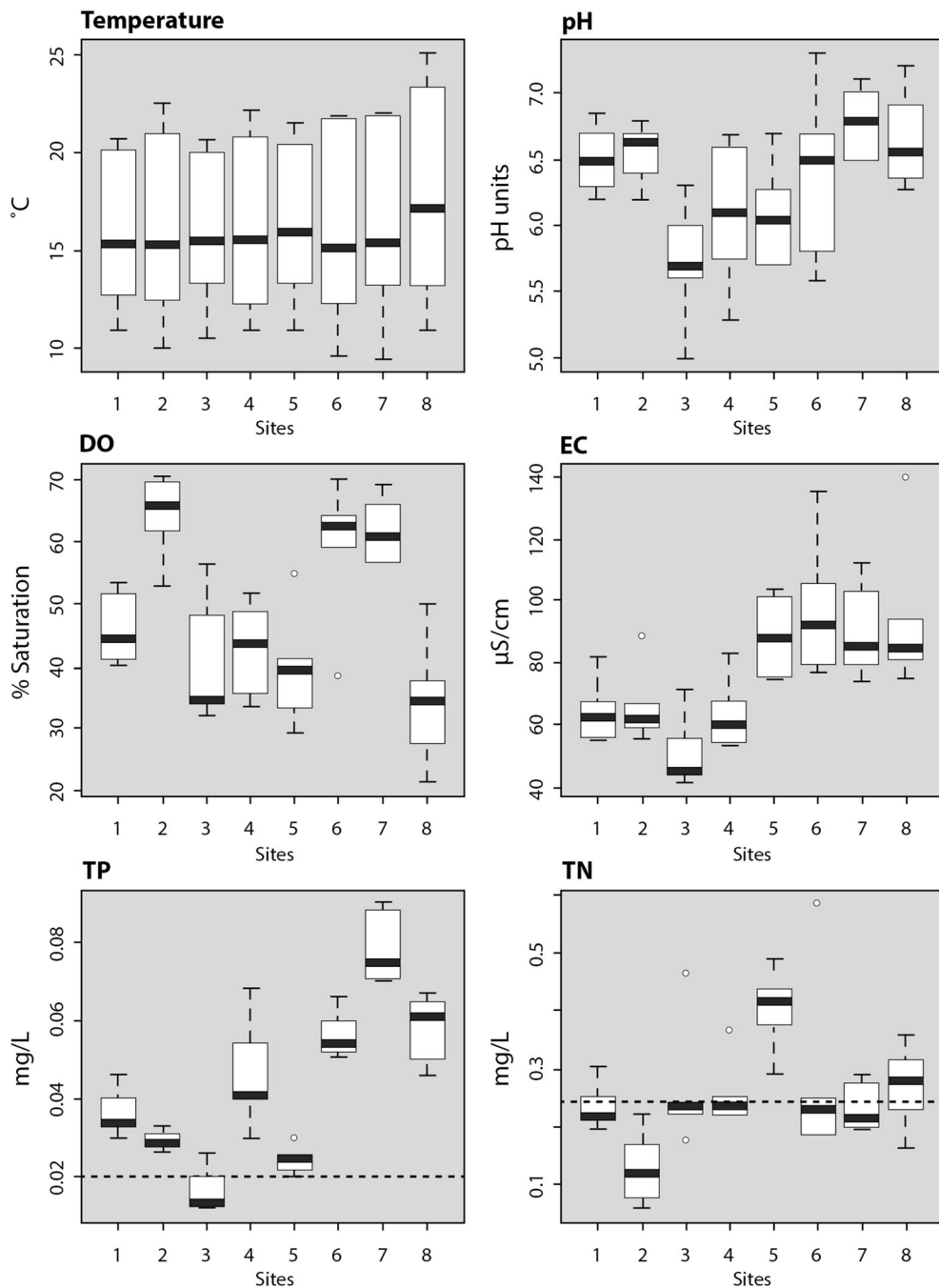


Fig. 2 Box plot of selected environmental variables across the eight sampling sites of the Coopers Creek Catchment. Box and whisker diagrams show median, minimum, maximum, 25th and 75th percentiles of values for samples in each group

CCA of these variables indicated they explained 30.5 % of the variation in the diatom data. Variance partitioning indicated that 42 % of the variation in the diatom data was due to these environmental variables

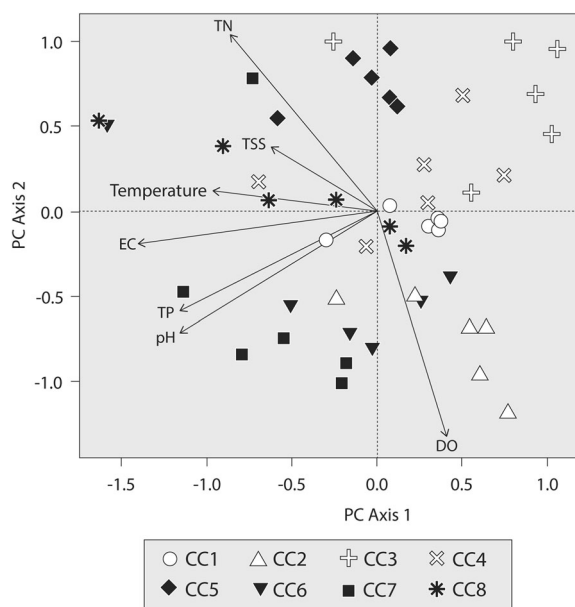
alone and the total interaction between them was 11.6 % (Fig. 6). EC explained the most variation (12.62 %), followed by TP (10.54 %), pH (7.65 %) and TN (4.98 %).

Table 1 Selected environmental data of the Coopers Creek Catchment during six sampling periods from March to August 2014

Sites	Temperature (°C)	pH	DO (% sat)	EC (µS/cm)	TSS (mg/l)	TP (mg/l)	TN (mg/l)
1							
Mean	15.9	6.51	45.80	64.5	1.75	0.036	0.233
Median	15.4	6.50	44.35	63.0	2.00	0.034	0.216
Min	10.9	6.20	40.20	55.0	0.50	0.030	0.196
Max	20.7	6.84	53.40	82.0	3.50	0.046	0.304
SD	4.0	0.25	5.54	9.9	1.13	0.006	0.040
2							
Mean	16.1	6.56	64.30	65.7	1.50	0.030	0.128
Median	15.3	6.64	65.65	61.5	0.50	0.029	0.117
Min	10.0	6.20	52.70	56.0	0.50	0.027	0.060
Max	22.6	6.80	70.50	89.0	4.50	0.033	0.224
SD	4.9	0.22	6.76	12.0	1.67	0.002	0.061
3							
Mean	15.9	5.71	40.02	50.8	5.42	0.016	0.261
Median	15.5	5.69	35.00	46.0	3.25	0.014	0.235
Min	10.5	5.00	31.70	42.0	0.50	0.012	0.176
Max	20.7	6.30	56.20	71.0	18.00	0.026	0.463
SD	4.0	0.44	9.87	11.0	6.43	0.006	0.101
4							
Mean	16.3	6.09	42.73	63.0	4.33	0.046	0.256
Median	15.6	6.10	43.60	60.0	2.00	0.041	0.238
Min	10.9	5.30	33.40	53.0	0.50	0.030	0.223
Max	22.2	6.70	51.60	83.0	17.00	0.068	0.364
SD	4.5	0.54	7.14	11.2	6.24	0.013	0.054
5							
Mean	16.3	6.08	39.62	88.2	3.83	0.024	0.403
Median	15.9	6.05	39.50	87.5	2.75	0.025	0.413
Min	10.9	5.70	29.40	74.0	0.50	0.020	0.290
Max	21.4	6.70	54.90	104.0	8.50	0.030	0.486
SD	4.1	0.38	8.68	12.8	3.06	0.003	0.068
6							
Mean	16.0	6.40	59.35	96.7	4.25	0.056	0.278
Median	15.2	6.50	62.30	92.0	3.50	0.054	0.232
Min	9.6	5.58	38.50	77.0	2.00	0.051	0.185
Max	21.9	7.30	69.90	135.0	7.50	0.066	0.583
SD	5.0	0.63	10.85	22.1	2.60	0.006	0.152
7							
Mean	16.2	6.78	61.63	89.7	9.85	0.078	0.232
Median	15.4	6.79	60.80	85.0	9.00	0.075	0.214
Min	9.5	6.50	56.40	74.0	2.50	0.070	0.197
Max	22.0	7.10	69.20	112.0	19.00	0.090	0.288
SD	5.0	0.25	5.70	14.9	7.16	0.009	0.040
8							
Mean	17.8	6.64	34.23	93.2	6.80	0.058	0.267
Median	17.2	6.55	34.35	84.5	6.50	0.061	0.277

Table 1 continued

Sites	Temperature (°C)	pH	DO (% sat)	EC (µS/cm)	TSS (mg/l)	TP (mg/l)	TN (mg/l)
Min	11.0	6.30	21.60	75.0	0.80	0.046	0.160
Max	25.1	7.20	49.70	140.0	16.00	0.067	0.354
SD	5.5	0.36	9.65	23.8	5.31	0.009	0.071
Sites 1–8							
Mean	16.3	6.35	48.46	76.5	4.72	0.043	0.257
Median	16.0	6.40	48.40	75.0	2.50	0.041	0.233
Min	9.5	5.00	21.60	42.0	0.50	0.012	0.060
Max	25.1	7.30	70.50	140.0	19.00	0.090	0.583
SD	4.3	0.50	13.31	21.7	5.07	0.021	0.103
Sites 1–4							
Mean	16.0	6.22	48.21	61.0	3.25	0.032	0.219
Median	15.6	6.30	47.25	59.5	2.00	0.031	0.223
Min	10.0	5.00	31.70	42.0	0.50	0.012	0.060
Max	22.6	6.84	70.50	89.0	18.00	0.068	0.463
SD	4.1	0.50	11.97	12.0	4.61	0.013	0.084
Sites 5–8							
Mean	16.6	6.47	48.71	91.9	6.18	0.054	0.295
Median	16.3	6.50	52.30	84.5	4.50	0.056	0.264
Min	9.5	5.58	21.60	74.0	0.50	0.020	0.160
Max	25.1	7.30	69.90	140.0	19.00	0.090	0.583
SD	4.7	0.48	14.79	18.0	5.18	0.021	0.109

Mean ($n = 6$)**Fig. 3** Plot of principal components analysis for selected environmental variables and sampling sites of the Coopers Creek Catchment

Diatoms

A diverse assemblage of diatom species was recorded from Coopers Creek Catchment (full dataset in supplementary information). There were 33 genera identified with a total of 98 species. Species diversity, determined by Shannon's diversity index, did not differ significantly between the Upper (2.2205) and Lower (2.3735) Catchment sites as demonstrated by mean of their probability of significance ($P = 0.27$). Site 2 recorded the highest diatom diversity (Table 3; mean 2.7396), while Site 4 recorded the lowest diatom diversity (mean 1.5595). There were 13 species (relative abundance >5 %) recorded at all sites across the catchment: *Achnanthes fagedii* Håkansson, *Achnanthes saxonica* Krasske ex Hustedt, *Bacillaria paradoxa* Gmelin, *Eunotia pirla* Carter & Flower, *Frustulia rhomboides* (Ehrenberg) De Toni, *Gomphonema angustatum* (Kützing) Rabenhorst, *Gomphonema gracile* Ehrenberg, *Gomphonema parvulum* (Kützing) Kützing, *Gyrosigma angulatum* (Quekett) Griffith & Henfrey, *Navicula cincta* (Ehrenberg)

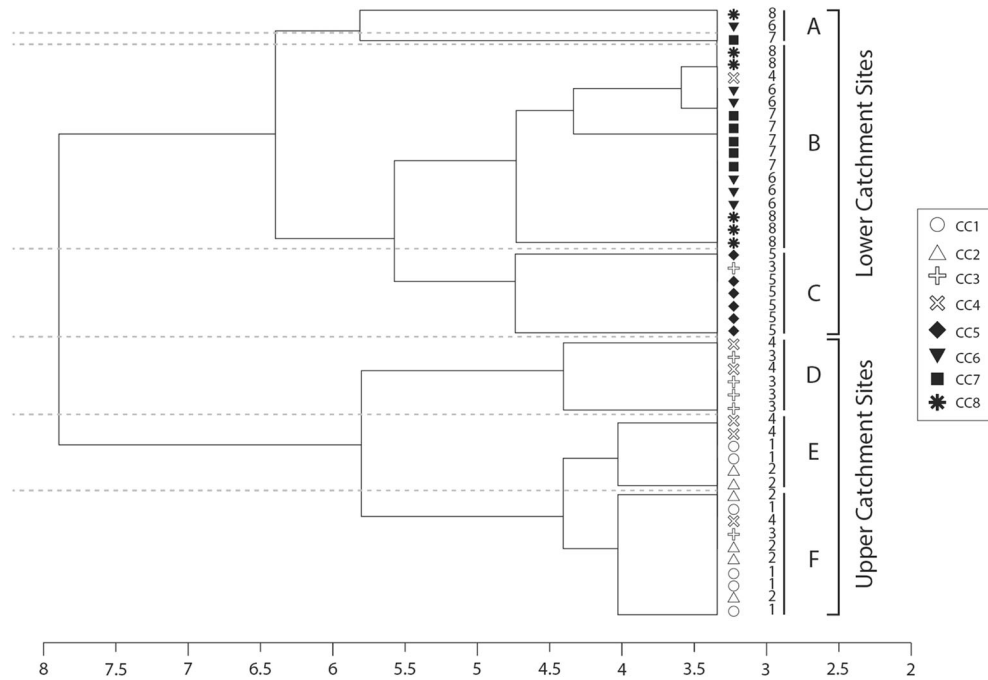


Fig. 4 Cluster analysis of selected environmental data of the sampling sites of the Coopers Creek Catchment during six sampling periods

Table 2 Canonical Correspondence Analyses of (a) all the environmental variables and (b) forward selected variables only (i.e., EC, TP, pH)

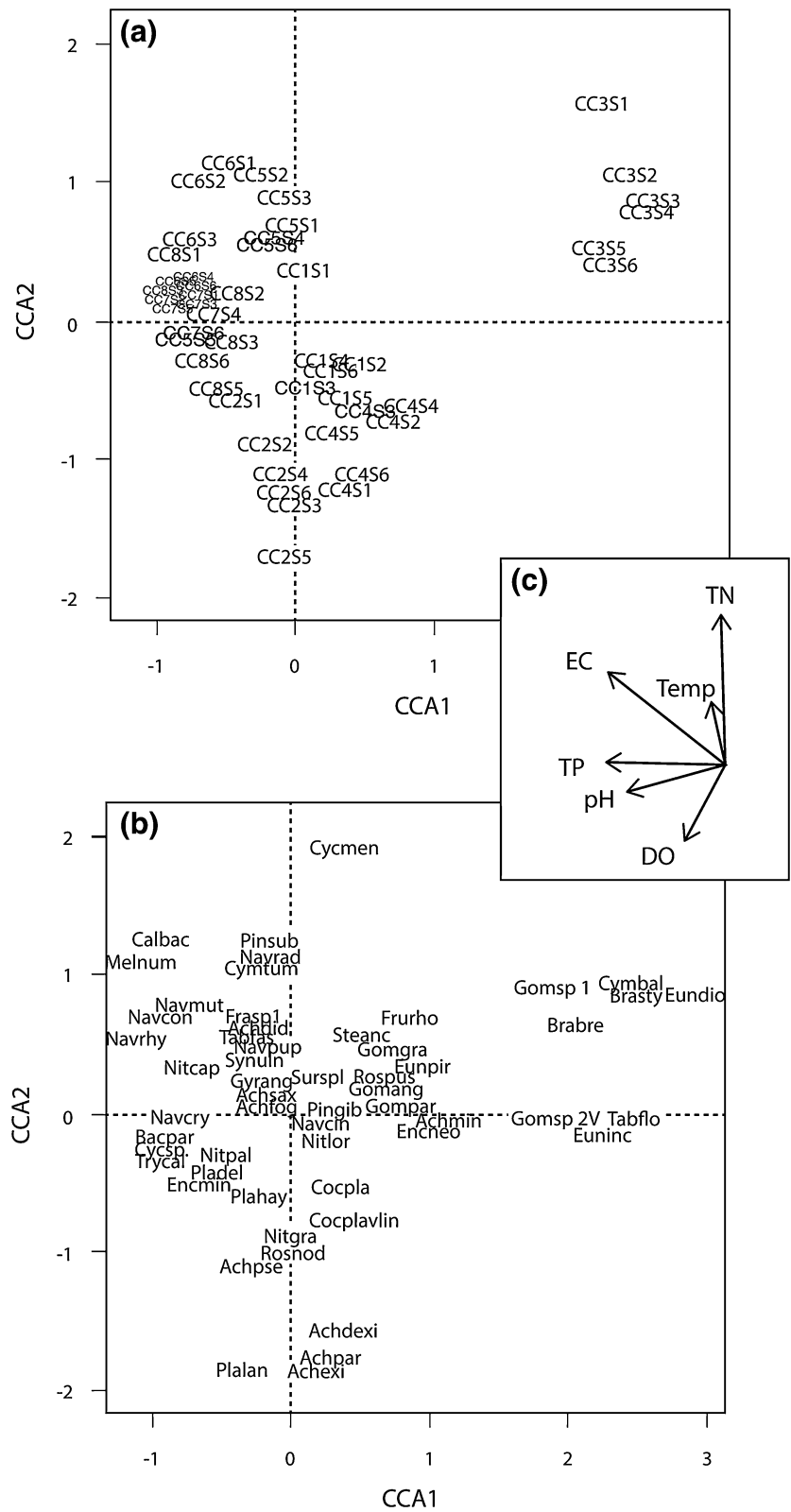
Axis	1	2	3	4
(a) All variables				
Eigenvalues	0.38213	0.11234	0.07569	0.05114
Σ Canonical eigenvalues	0.6885			
Σ All eigenvalues	1.9401			
(b) Forward selected variables				
Eigenvalues	0.31634	0.10789	0.06455	0.05251
Σ Canonical eigenvalues	0.5917			
Σ All eigenvalues	1.9401			
Σ Sum				

Ralfs, *Nitzschia capitellata* Hustedt, *Nitzschia palea* (Kützing) Smith and *Planothidium haynaldii* Schaarschmidt. The diatom communities were generally dominated by three–five species (relative abundance >20 %) at each site.

Community composition varied across sites with a distinction between dominant species in the Upper and Lower Catchments (Fig. 7). In the Upper Catchment, Sites 1 and 2 show similar diatom community composition (Figs. 5, 7) with the dominant species *P. haynaldii*, *N. cincta*, *G. angulatum*, *N. palea*, *A. fagedii* and *Cocconeis placentula* var *lineata*

(Ehrenberg) van Heurck. The community composition of Sites 3 and 4 are quite different to Sites 1 and 2 and each other. The dominant species at Site 3 were *Gomphonema spec 2* (Vyverman 1995) and *Tabellaria flocculosa* (Roth) Kützing, neither of which were identified as being significant in the other Upper or Lower Catchment sites. *G. angustatum* was another dominant species at this site and was recorded at all sites. Site 4 was dominated by three species, *C. placentula* var *lineata*, *C. placentula* Ehrenberg and *G. angustatum*. *G. spec 2* showed a negative correlation ($r^2 = -0.50$) with TP (Fig. 8). The four most dominant

Fig. 5 Canonical Correspondence Analysis of the dataset **a** sites displayed, **b** species displayed and **c** environmental parameters displayed. *DO* dissolved oxygen, *TP* total phosphorus, *EC* electrical conductivity, *TSS* total suspended solids, *Temp* temperature, *TN* total nitrogen



species of the Upper Catchment were *C. placentula* var *lineata*, *C. placentula*, *G. spec 2* and *T. flocculosa*.

In the Lower Catchment Site 5 was dominated by *A. saxonica*, *A. fogedii* and *N. cincta*. Sites 6–8 had similar diatom community composition with *B. paradoxa*, *Navicula cryptocephala* Kützing, *N. palea* and *P. haynaldii* identified as the dominant species at all three sites (Fig. 7). Three species, *Navicula mutica* var *mutica* Kützing, *Navicula constans* Hustedt and *N. capitellata*, were identified as being >5 % relative abundance at all sites in the Lower Catchment. *B. paradoxa* (r^2 0.65) and *N. constans* (r^2 0.51) had a positive correlation with TP, while *N. constans* (r^2 0.62) and *N. capitellata* (r^2 0.62) showed positive correlations with EC (Fig. 8). The four most dominant species of the Lower Catchment were *B. paradoxa*, *N. cryptocephala*, *N. mutica* var *mutica* and *A. fogedii*.

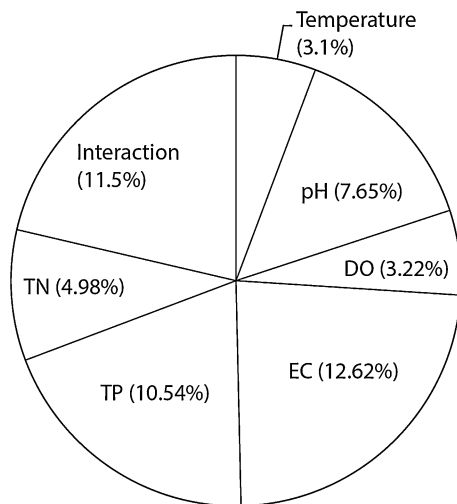


Fig. 6 Summary of variance partitioning results

Table 3 Pearson species diversity of the Coopers Creek Catchment

Sample periods	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	Site 7	Site 8
1	2.4267	3.2584	2.1995	1.2380	2.0813	2.8770	2.7338	2.6994
2	2.4267	2.4963	1.4946	1.7594	2.9146	2.4894	2.5916	1.4721
3	2.4532	2.8523	1.6594	1.6927	2.5759	2.5020	2.6252	2.4675
4	2.7300	3.0405	2.3445	1.5176	2.7698	2.2860	2.4277	2.5288
5	2.6008	2.3423	2.2313	1.7037	2.6293	2.0902	2.2787	2.0946
6	2.8551	2.4478	2.0767	1.4453	2.0631	2.2372	1.4729	2.0569
Mean	2.5821	2.7396	2.0010	1.5595	2.5056	2.4136	2.3550	2.2199
Upper Catchment mean	2.2205							
Lower Catchment mean	2.3735							

The ecological tolerances and common diatom index values of the dominant species of the Coopers Creek Catchment are summarised in Table 4.

Discussion

Physicochemical data

Natural processes such as rainfall, flood events, functional riparian zones and seasonal variations in temperature contribute to water quality changes and seasonal variation (Singh et al., 2005; Gay & Ferguson, 2012). Anthropogenic activities such as land clearing and intensive agriculture may cause a decline in water quality (Aplin et al., 1999; Smith et al., 1999; Perna & Burrows, 2005; Dudgeon et al., 2006; Dodds & Whiles, 2010). There was little seasonal variability captured in this study because sampling was conducted across a dry year; hence most variability in the dataset could be attributed to anthropogenic impacts and catchment characteristics. PCA analysis showed that the Lower Catchment sites were more strongly influenced by the measured environmental variables. The cluster analysis demonstrated Upper and Lower sites are different. This may be attributed to greater anthropogenic influence and more intensive land use activities contributing to poorer water quality in the Lower Catchment. The environmental parameters measured, particularly nutrient loads, were specifically selected because they are strong indicators of anthropogenic influence, meeting the project aims.

The distinction between Upper and Lower sites was evident and is indicative of the intensive agricultural practices of the Lower Catchment influencing both

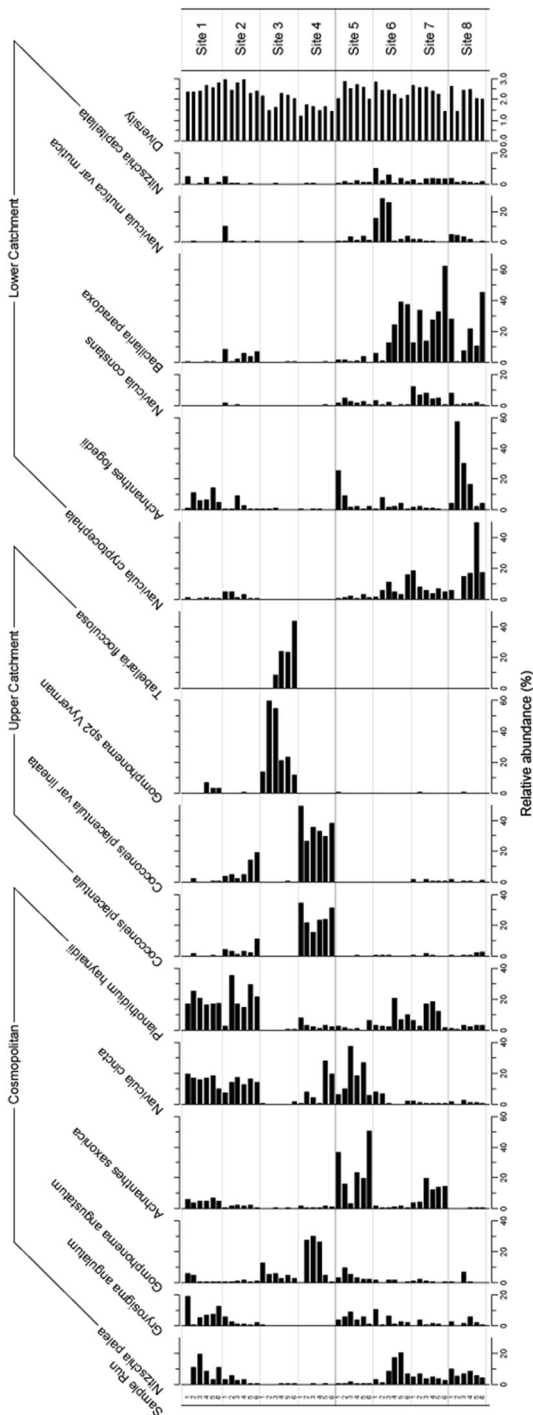


Fig. 7 Dominant diatom species of eight sample sites of the Coopers Creek Catchment

higher nutrient levels and EC. TP concentrations exceeded the ANZECC (2000) trigger value (0.020 mg/l) for freshwater streams by as much as

4.5 times and TN almost two times at some sites. The data in this study did not indicate the origins of phosphorus; though they can generally be attributed to the Catchment's geology and soils derived from volcanic activity of the Mount Warning Volcano (Morand, 1994; Rous Water 2009a, b). Fertiliser additions from land use, particularly macadamia plantations, are sources of higher nutrients in the Lower Catchment and likely responsible for the greatest effect on water quality (Rous Water, 2009a). EC results ($P = 8.87E-09$) clearly demonstrate a differentiation between Upper (mean 61 $\mu\text{S}/\text{cm}$) and Lower (mean 91.9 $\mu\text{S}/\text{cm}$) management areas. Generally lower river reaches have higher conductivity though these results represent a significant increase. They are also indicative of the surrounding land use activities with greater anthropogenic influence in the Lower Catchment. The low pH values recorded may be influenced by the acidity of the Catchment's geology and soils derived from volcanic activity of the Mount Warning Volcano (Morand, 1994; Rous Water, 2009a, b). The acidifying effect of nitrogen fertilisers used heavily in macadamia production may also be a heavy contributor (Morand, 1994).

CCA and variance partitioning showed that EC, TP and pH were the most important variables in explaining the variation in diatom composition and it is these parameters that are generally selected for evaluation of ecological tolerances in the common diatom indices. There was a significant amount of variation in the species dataset not explained by measured parameters and hence further research needs to be conducted to explain the main determining factors in the diatom community.

Diatom diversity and community composition

The Coopers Creek Catchment showed a high level of species diversity compared to riverine studies of a similar scale in both Australia and other sub-tropical regions (Blinn & Bailey, 2001; Bellinger et al., 2006; Newall et al., 2006). Diversity was slightly higher in the Lower Catchment, however, the difference was not significant ($P = 0.27$). This diversity pattern is consistent with the intermediate disturbance hypothesis (Connell, 2002) which suggests that higher diversity is maintained under intermediate scales of disturbances. Our result is similarly consistent with other studies (Chessman, 1986; Blinn & Bailey, 2001; Sonneman

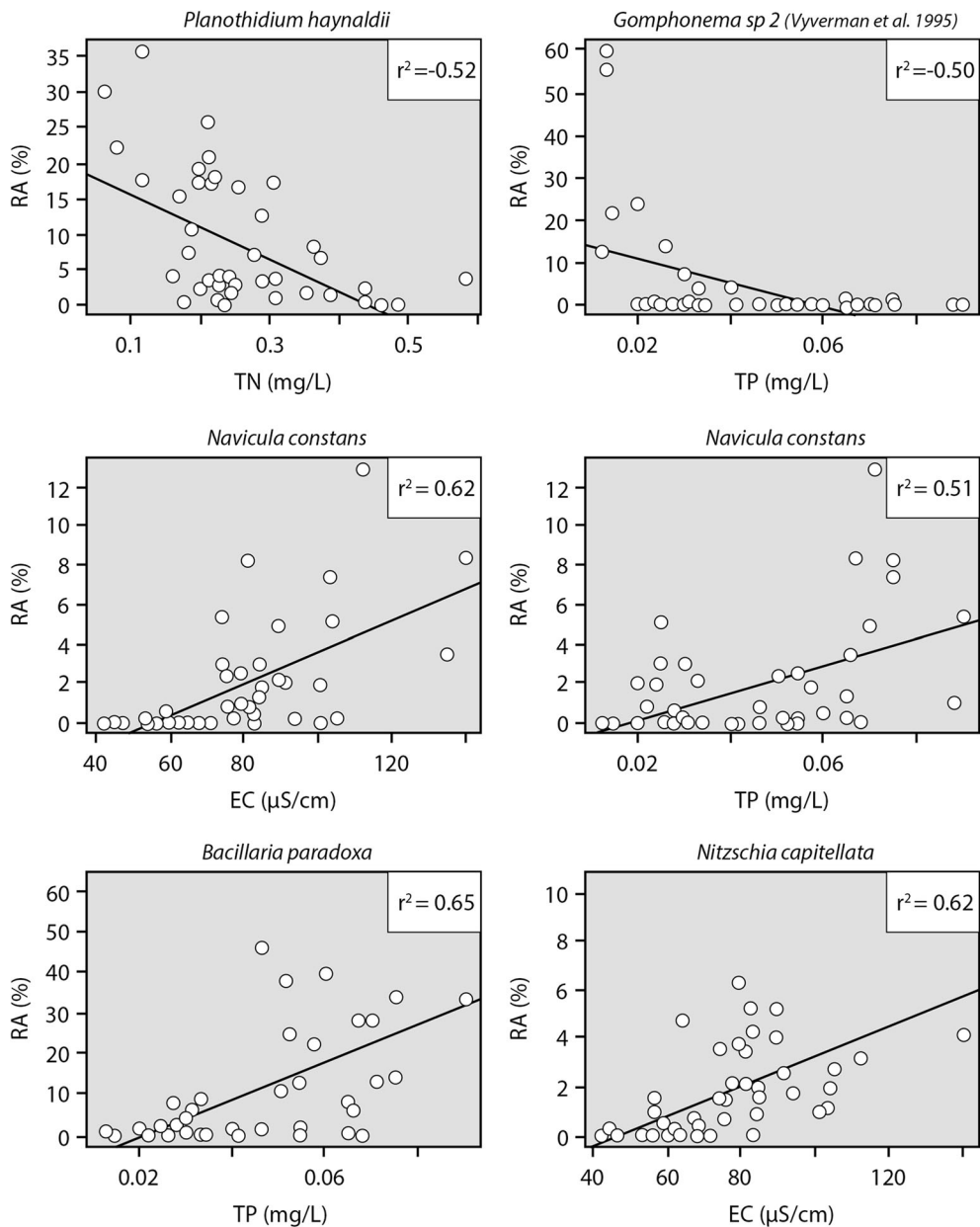


Fig. 8 Correlation of dominant diatom species and environmental variables ($r^2 > 0.5$)

et al., 2001; Bellinger et al., 2006) which found lower or no significant difference in species diversity in less impacted or undisturbed streams. They are also consistent with the findings of Stenger-Kovács et al. (2014) who found a linear relationship between diversity and stream order where diversity increased by 10 % per unit of stream order. Stenger-Kovács et al. (2014) suggest that these results could be

explained by geological differences and higher nutrient loading in higher order streams as land use becomes more extensive and intensive as stream order increases.

Community composition was similar throughout Sites 1, 2 and 4 of the Upper Catchment and within all sites of the Lower Catchment (Figs. 5, 7). PCA showed that Site 3 was very different from all the

Table 4 Ecological preferences of dominant species of the Coopers Creek Catchment with common diatom index values

Species	Salinity	pH	TP (mg/l ²)	Trophic status/ pollution tolerance	DIAR	DSIAR	PTI	TDI	IPS
<i>Achnanthes fagedii</i>	Brackish				4				
<i>Achnanthes saxonica</i>	Fresh–brackish	7	<0.01	Ultra oligotrophic mild pollution	4	69		1	4
<i>Bacillaria paradoxa</i>	Brackish	>7	0.1–0.35	Eutrophic Heavy pollution	2	32	2	5	2
<i>Cocconeis placentula</i>	Fresh–brackish	>7	0.035–0.1	Eutrophic Moderate pollution	2	33	3	3	4
<i>Cocconeis placentula</i> var <i>lineata</i>		>7	0.035–0.1	Eutrophic Very pollution sensitive	2			3	5
<i>Gomphonema angustatum</i>	Freshwater		<0.01	Moderate nutrients	6	37	2	1	3
<i>Gomphonema spec 2</i>					6				
<i>Gyrosigma angulatum</i>					3				
<i>Navicula cincta</i>	Fresh–brackish	>7	0.1–0.35	Polytrophic Moderate pollution	3	52	2	4	3
<i>Navicula constans</i>	Fresh–brackish				3		4		
<i>Navicula cryptocephala</i>	Fresh–brackish	7	0.1–0.35	Hypertrophic High nutrients	3	46	3	4	4
<i>Navicula mutica</i> var <i>mutica</i>					3		2		
<i>Nitzschia capitellata</i>	Brackish	>7	0.1–0.35	Hypertrophic Very heavy pollution	1	55		4	1
<i>Nitzschia palea</i>	Fresh–brackish	7	0.35–1.0	Hypertrophic Very heavy pollution	1	49	1	5	1
<i>Planothidium haynaldii</i>						49			
<i>Tabellaria flocculosa</i>	Freshwater	<7	0.01–0.035	Oligotrophic, very pollution sensitive	10	77		2	5

Sources Kelly & Whitton (1995), Chessman et al. (1999, 2007), Gell et al. (1999), Sonneman et al. (2000), Kelly et al. (2001, 2005), Muscio (2002), Yu et al. (2004), Kelly & Yallop (2012) and Grudzinska et al. (2014)

DIAR Diatom Index of Australian Rivers (values 1: low sensitivity to 10: high sensitivity), DSIAR Diatom species Index of Australian Rivers (values 1: low sensitivity to 100: high sensitivity), PTI Pollution Tolerance Index (values 1: tolerant to 4: sensitive), IPS Indice de Polluosensibilité (values 1: very tolerant to 5: very sensitive), TDI Trophic Diatom Index (1: very sensitive to 5: very tolerant)

other sites in diatom community composition and had little relationship with the environmental variables measured. This site had the least anthropogenic influence of all sites with all areas upstream in protected areas. The physical and chemical parameters measured may not fully capture the factors influencing community composition (Kelly et al., 2001; Chessman et al., 2007). CCA results showed that 35.48 % of the species variation was explained by the measured environmental factors across the catchment, suggesting other variables may be the key to understanding community composition and relationships to environment, particularly at Site 3. Considering the land use of

the catchment, organic pollution, trace metals and toxic chemicals such as pesticides, may be playing a greater role in community composition in the Lower Catchment sites (Kelly et al., 2001).

Key indicator species

Community composition of the Upper Catchment was dominated by four species, *C. placentula*, *C. placentula* var *lineata*, *G. spec 2* and *T. flocculosa* which were not evident in the Lower Catchment (Fig. 7). *G. spec 2* and *T. flocculosa* are known as having low to medium tolerance to common anthropogenic

stressors, while *C. placentula* and *C. placentula* var *lineata* have been reported as having varying degrees of tolerance to eutrophication and organic pollution in the common diatom indices and literature (IPS: very sensitive, TDI and PTI: moderate, DSIAR and DIAR: very tolerant; Table 4). However, these two species were predominantly found in the Upper Catchment and so along with *G. spec 2* and *T. flocculosa*, were designated as key indicator species of moderate health in the Coopers Creek Catchment. Four species: *B. paradoxa*, *N. cryptocephala*, *N. mutica* var *mutica* and *A. fogedii*, were dominant in the Lower Catchment. These species generally show greater tolerance to high nutrient concentrations and pollution (Table 4) and were considered the key indicator species of poor health in the Coopers Creek Catchment.

Species with significant correlations with individual environmental variables can also be considered as key indicator species (Cooper, 2001; Potapova et al., 2004; Weilhoefer & Pan, 2007). In the Upper Catchment, *G. spec 2* had a negative correlation ($r^2 = -0.50$) with TP. Limited information has been recorded on the ecological tolerance of *G. spec 2*, though the *Gomphonema* genus is generally tolerant of moderate pollution (Chessman et al., 1999). However, results of this study indicated sensitivity of this species to TP, as evidenced by its high abundance in sites with lower concentrations of TP, particularly Site 3.

Contradictions are generally known to occur in the literature of diatom ecology with regard to ecological preferences of species. Research suggests that ecological tolerances of diatom species may also differ across climatic regions and natural variations in water quality (Kelly et al., 2005; Bellinger et al., 2006; Philibert et al., 2006; Bere & Tundisi, 2011; Besse-Lototskaya et al., 2011; Tan et al., 2013). This questions the applicability of Northern Hemisphere and temperate region indices for use in sub-tropical regions of Australia (Newall et al., 2006; Chessman et al., 2007; Tan et al., 2013) and also highlights the need for more research into the ecological tolerance of diatoms in both Australia and internationally (Hermany et al., 2006; Lobo et al., 2010; Besse-Lototskaya et al., 2011). Taxonomic uncertainty is also an issue that needs further attention as misidentification of diatom species is not unusual and can lead to errors in assemblage–environment relationships and subsequent misrepresentation of the trophic status of a river

system (Lobo et al., 2010; Besse-Lototskaya et al., 2011; Rimet & Bouchez, 2012).

In the Lower Catchment, *B. paradoxa* was dominant at sites with the highest TP concentrations. Research suggests this species is a strong indicator of anthropogenic influence due to its association with high P concentrations (Table 4) (Chessman, 1986; Sonneman et al., 2000; Blinn & Bailey, 2001; Kelly et al., 2005; Dela-Cruz et al., 2006). Little ecological information is available for *N. constans* which was identified as correlating with TP. *N. capitellata* and *N. constans* showed positive correlations with EC. Research indicates that *N. capitellata* is a strong indicator of heavy pollution (Table 4) which supports the results of this study (Sonneman et al., 2000; Kelly et al., 2005; Dela-Cruz et al., 2006). Bahls et al. (1985) reported that *P. haynaldii* had low tolerance to TN concentrations over 0.3 mg/l. This observation was reflected in our results suggesting *P. haynaldii* may be a good indicator of low TN concentrations. These species/environmental variable relationships are consistent with land use activities and level of anthropogenic influence and are considered the key indicator species of individual environmental variables in the catchment.

Diatom indices

Diatoms are increasingly used as bio-indicators of water quality, particularly with regard to anthropogenic stressors impacting biological integrity and ecosystem health (Reid et al., 1995; Kelly et al., 1998, 2009b; Chessman et al., 1999, 2007; Gómez & Licursi, 2001; Li et al., 2010; Bere & Tundisi, 2011; Tan et al., 2013). However, as most of the research and indices developed have been centred on temperate regions, they may not be applicable in a sub-tropical environment in Australia (Newall et al., 2006; Chessman et al., 2007).

The results of this study have revealed the need to develop catchment-based calibration sets or indices, as many species identified in this study did not appear in the DSIAR or commonly used indices of the Northern Hemisphere (DSIAR 50 %, PTI 32 % and TDI 26 %) (Kelly & Whitton, 1995; Muscio, 2002; Chessman et al., 2007; Kelly & Yallop, 2012). Only 14 % of diatom species in this study were included in the ‘reliable diatom taxa list’ developed by Besse-Lototskaya et al. (2011), which evaluated the European

diatom trophic indices. This seems to be a common occurrence. Salomoni et al. (2011) found that the Water Quality Biological Index (WQBI; Lobo et al., 2004) developed for rivers in the southern Brazilian Region, was not adequate and subsequently developed a Gravataí WQBI to reflect local environmental characteristics. Research also suggests that the performance of some indices is proportional to the number of species included in the index applied, with lower proportions resulting in lower performance of the index (De la Rey et al., 2004; Newall et al., 2006; Besse-Lototskaya et al., 2011; Tan et al., 2013). Based on the results of the Coopers Creek study, it may be feasible to construct a diatom index suitable for sub-tropical rivers in Australia which would include a greater number of species, increasing the effectiveness of the potential index for use by management agencies.

Conclusion

The use of diatoms as bio-indicators of water quality is well developed in the Northern Hemisphere; however, it is an underdeveloped science in Australia with few diatom river indices available, and all developed in temperate climate zones. Many of the species identified in this study did not appear in the DSIAR or other commonly used indices of the Northern Hemisphere. This study showed that diatoms have potential value as bio-indicators of sub-tropical river health with several species identified as indicators of water quality. It has also revealed the need for expansion of this project, spatially and temporally, to other sub-tropical catchments to build a full dataset for the region.

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