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APPLIED ISSUE

Periphyton-based transfer functions to assess ecological imbalance and management of a subtropical ombrotrophic peatland

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SUMMARY

1. To assess the biological status and response of aquatic resources to management actions, managers and decision-makers require accurate and precise metrics. This is especially true for some parts of the Florida Everglades where multiple stressors (e.g. hydrologic alterations and eutrophication) have resulted in a highly degraded and fragmented ecosystem. Biological assessments are required that directly allow for the evaluation of historical and current status and responses to implantation of large-scale restoration projects.
2. Utilising periphyton composition and water-quality data obtained from long-term (15 years) monitoring programmes, we developed calibrated and verified periphyton-based numerical models (transfer functions) that could be used to simultaneously assess multiple stressors affecting the Everglades peatland (e.g. salinity, nitrogen and phosphorus). Periphyton is an ideal indicator because responses to stressors are rapid and predictable and possess valued ecological attributes.
3. Weighted averaging partial least squares regression was used to develop models to infer water-quality concentrations from 456 samples comprising 319 periphyton taxa. Measured versus periphyton-inferred concentrations were strongly related for log-transformed salinity ($r^2_{\mathrm{jackknife}}$ = 0.81; RMSEP 0.15 mg L^{-1}) and log-transformed total phosphorus (TP; $r^2_{\mathrm{jackknife}}$ = 0.70; RMSEP 0.18 mg L^{-1}), but weakly related for total Kjeldahl nitrogen (TKN) ($r^2_{\mathrm{jackknife}}$ = 0.46; RMSEP 0.12 mg L^{-1}). Validation results using an independent 455 sample data set were similar (log(salinity) r^2 = 0.78, log(TP) r^2 = 0.65 and log(TKN) r^2 = 0.38).
4. Water Conservation Area 1 (WCA-1), a large ombrotrophic subtropical peatland impacted by multiple water-quality stressors that has undergone major changes in water management, was used as a case study. The models were applied to a long-term periphyton data set to reconstruct water-quality trends in relation to restoration efforts to reduce nutrient loading to the Everglades. The combination of biologically inferred TP and salinity was used to identify the ecological status of periphyton assemblages. Periphyton assemblages were ecologically imbalanced with respect to salinity and TP. Salinity imbalance varied spatially and temporally, whereas TP was spatially restricted. Imbalances caused by water management were owing to salinity more so than to TP.
5. The transfer functions developed for the Everglades are trait-based quantitative numerical methods and are ideal because the abundances of species are modelled numerically in relation to a stressor. The resulting inferred value is a numerical representation of the stressor's effect on biological condition that can be compared against the management of the stressor independent of other factors. The benefits are that biological lags or hysteresis events can easily be identified and environmental conditions can be estimated when measurements are lacking. Reporting biological

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assessments in terms of well-defined water-quality metrics (e.g. numeric criterion) increased the communicative ability of the assessment. The use of multiple metrics to assess ecological imbalance increased the ability to identify probable causes.

Keywords: assessment, Everglades, phosphorus, salinity, wetland eutrophication

Introduction

To assess the biological status and response of aquatic resources to management actions, managers and decision makers require accurate and precise metrics. Key features of an ideal metric are that it be an early warning indicator of environmental stress or impacts, broadly applicable across space and time, reflective of the causal factor and existing degradation, easily interpretable and directly related to management goals and cost-effective (Carignan & Villard, 2002; Marchant, Norris & Milligan, 2006). It is also important to define whether the indicator measures stressors or biological condition (Stevenson *et al.*, 2008). Stressors focus on the protection and restoration of valued ecological attributes from habitat alterations and contaminants, whilst biological condition captures natural capital and ecosystem services.

Periphyton, a consortium of algae, bacteria, fungi and invertebrates imbedded within an organic and inorganic matrix, is a commonly used sensitive biological indicator of water quality because responses to stressors are rapid and predictable (McCormick & Stevenson, 1998; Hill *et al.*, 2000; Stevenson, 2001; Gaiser, 2009). Since responses occur at both the structural (i.e. taxonomic composition) and functional (i.e. taxonomic sensitivity, chemistry and production) levels, periphyton indices of biological integrity (PIBI) are prime for development (Hill *et al.*, 2000; Gaiser, 2009). Development of periphyton-based methods has primarily utilised diatoms to assess conditions or the integrity of streams (Dixit & Smol, 1994; Stevenson *et al.*, 2008) and wetlands (Lane, 2007; Gaiser & Rühland, 2010). Assessments that incorporate soft algae are, however, less common (Hill *et al.*, 2000; King, Barker & Jones, 2000; Munn, Black & Gruber, 2002; DeNicola *et al.*, 2004; Gaiser, 2009). However, such identity-based metrics are not suited for assessing management practices because they do not provide a mechanistic linkage between the stressor and biota (Pollard & Yuan, 2010; Culp *et al.*, 2011).

In their review of ecological integrity assessment, Dolédec & Statzner (2010) advocate predictive methods that quantify differences in ecological integrity between expected and observed conditions. Methods based on biological and physiological characteristics, often called trait-based measures, are most useful because they do provide a mechanistic link (Pollard & Yuan, 2010; Culp *et al.*, 2011). If species are distributed along environmental gradients, then numerical methods (e.g. transfer functions) have potential as a predictive means to assess ecological integrity (Birks, 2010). Such indicator techniques have recently been modified from palaeoecological methods to infer present-day water-quality trends from modern day diatom assemblages (Philibert *et al.*, 2006; Ponader *et al.*, 2008; Wachnicka *et al.*, 2010). Transfer functions are procedures that produce calibrated quantitative estimates of an environmental variable (e.g. water quality) from proxy data such as biological assemblages (Guiot & de Vernal, 2007; Birks, 2010). These methods may be advantageous for environmental resource managers because the biological integrity end-result is reported in the same units as the stressor (i.e. concentration); thus, the metric is easily interpreted and communicated, especially when put in context of well-defined targets, benchmarks, thresholds or criteria. Numeric nutrient criteria are examples of well-defined targets, or benchmarks, since they establish the concentrations protective of designated uses of aquatic ecosystems.

The subtropical Florida Everglades peatland has been severely impacted by human activities that have altered marsh hydrology and degraded water quality, primarily in the form of phosphorus enrichment (Sklar *et al.*, 2005). Significant efforts are underway to restore the hydrology and reduce agricultural and urban nutrient loading (Sklar *et al.*, 2005). Biological assessment has been, and remains, a fundamental component of the management of the Everglades ecosystem. Periphyton-based metrics were used in the development of the total phosphorus (TP) numeric nutrient criterion (McCormick & Stevenson, 1998; Gaiser, 2009) and proposed for assessing ecosystem responses to management changes (Gaiser *et al.*, 2006; Gaiser, 2009; Wachnicka *et al.*, 2010). Eutrophication and, to a lesser extent, hydrology have been the primary focus of indicator development, but the Everglades is simultaneously influenced by other stressors that can complicate ecological assessments. These include, but are not limited to, mineral enrichment, nitrogen and sea-level rise (Wachnicka *et al.*, 2010; Newman & Hagerthey, 2011). This complication is minimised by many Everglades taxa having broad geographical ranges with well-established indicator status (e.g. Potapova & Charles, 2003, 2007). Moreover, the strong relationships between Everglades periphyton and multiple stressors (Gaiser *et al.*, 2011), along with well-defined targets or thresholds, suggest that predictive methods based on algal indicators could be developed to assess ecological responses to specific management and restoration actions within the Everglades.

The objective of this study was to develop an assessment tool to transform periphyton composition data collected in the course of environmental monitoring into a more meaningful metric of the ecological integrity of the Everglades peatland. The intent is to provide resource managers and policy makers with a tool for assessing biological responses to specific management and restoration actions. First, we develop, calibrate and verify periphyton-based numerical models (transfer functions) to simultaneously assess multiple stressors common to the Everglades peatland. Specifically, the stressors of interest were salinity, phosphorus and nitrogen. We use the term salinity to describe freshwater mineral content rather than the common analogues conductivity or total dissolved solids (TDS) because loads, flow-weighted means and mass balances are more readily calculated and ecologically meaningful. Second, since biological assessments are often comprised of large complex data sets that are difficult to synthesise and communicate to decision makers and stakeholders, we further improve the communicative and interpretive ability by employing a tricolour coding system to visually enhance trends in time. Third, we demonstrate the effectiveness of the numerical models to convey information required by decision makers using a case study of the Everglades impoundment, Water Conservation Area 1 (WCA-1). WCA-1 is impacted by multiple stressors (salinity and TP) and has undergone major infrastructural changes in efforts to reduce nutrient loading yet maintain hydrologic needs.

Methods

Periphyton data set

The analyses herein are based on periphyton taxonomic composition grown on artificial substrata, glass microscope slides contained within a periphytometer (Wildlife Supply Company, Buffalo, NY, U.S.A.). The Everglades periphytometer data set, maintained within a relational database that incorporates measures to ensure taxonomic verification, consistency and integrity, consists of 3800 samples collected between 1994 and 2009 from 151 monitoring sites and 105 experimental mesocosms. Geographically, the sites encompass the Everglades peatland ecosystem (Fig. 1). The monitoring intent varied among regions from assessing biological responses to eutrophication and hydrology to regulatory requirements; therefore, the sampling frequency and intensity varied among regions.

Periphytometers were deployed for 6–8 weeks. Upon collection, the glass slides were placed in Whirl-Pak® bags (NASCO, Fort Atkinson, WI, U.S.A.), transported to the laboratory on ice and stored at 4 °C until processed, usually within 24 h. Periphyton was removed, homogenised with a BioSpec BioHomogenizer (BioSpec Products Inc., Bartlesville, OK, U.S.A.) and preserved with 30% formalin. Taxonomic analysis was conducted by the Florida Department of Environmental Protection (FDEP) Biology Section (Tallahassee, FL, U.S.A.). Soft algal counts consisted of identifying to lowest taxonomic level a minimum of 300 viable cells in an Utermöhl cell using an inverted microscope at a magnification of 420×. The total number of diatoms was recorded in the soft counts, but identifications and proportional counts were made from a subsample cleaned with 2*N* nitric acid (HNO_3) and permanently mounted using Permount™ (ProSciTech, Kirwan, Qld, Australia). Approximately 500 valves were identified at a magnification of 1000× to the lowest taxonomic level. Cell densities (number cm^{-2}) and relative abundances (%) were calculated for each sample.

Water quality was also assessed at each site to varying degrees of frequency and parameter suites. At all sites, TP, total Kjeldahl nitrogen (TKN), pH, water temperature, dissolved oxygen and conductivity were typically sampled monthly. Two monitoring programmes involved a more robust suite of parameters that included dissolved calcium (Ca^{2+}), magnesium (Mg^{2+}), sodium (Na^{+}), potassium (K^{+}), alkalinity (A_T), sulphate (SO_4^{2-}), chloride (Cl^{-}), nitrate + nitrite (NO_x^{-}), iron (Fe^{2+}), silica (SiO_2^{4-}) and organic carbon (DOC). Analyses were performed by the South Florida Water Management District's (SFWMD) Chemistry Laboratory (West Palm Beach, FL, U.S.A.) or the FDEP Central Chemistry Laboratory (Tallahassee, FL, U.S.A.) using standard methods. Conductivity and pH were measured using calibrated Hydrolab MiniSondes or DataSondes (Hach Environmental Headquarters, Loveland, CO, U.S.A.). Salinity was calculated as the sum of dissolved Ca^{2+}, Mg^{2+}, Na^{+}, K^{+}, SO_4^{2-}, Cl^{-}, NO_x^{-}, Fe^{2+}, SiO_2^{4-} and bicarbonate–carbonate (HCO_3^{-}) (Wetzel, 2001), where HCO_3^{-} was calculated from A_T and pH (Stumm & Morgan, 1996); however, if paired pH values were not available, we estimated HCO_3^{-} from linear regression of A_T ($r^2 = 0.99$; $y_0 = 1.2$; $a = 0.0584$). Alternatively, if salinity

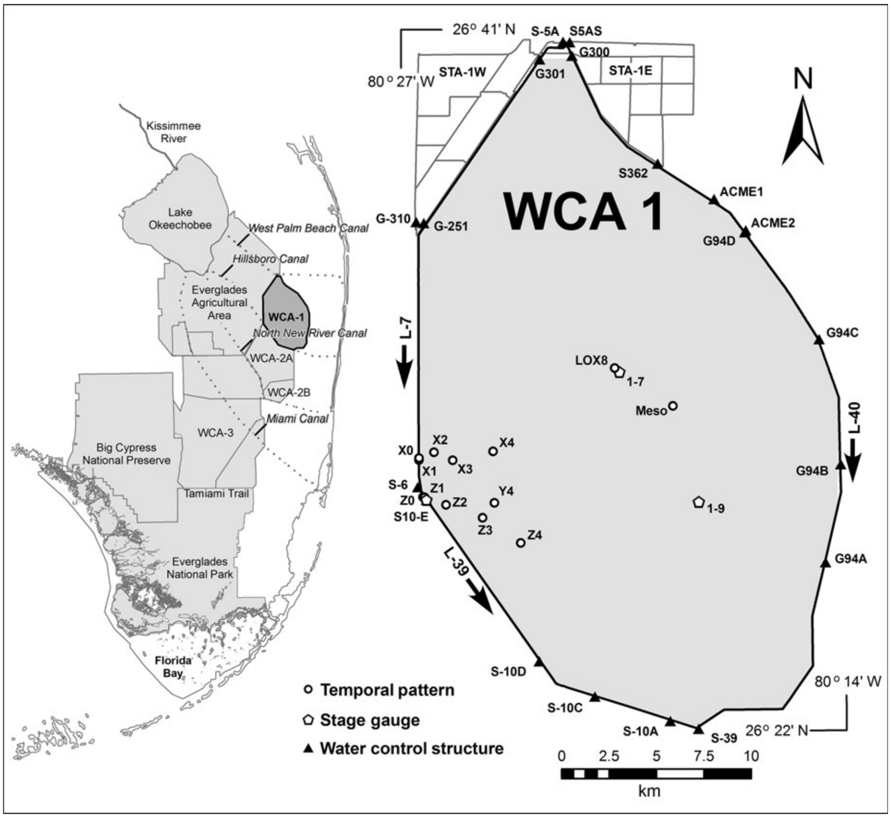

Fig. 1 Map of Water Conservation Area 1 within the Everglades landscape. The long-term monitoring and synoptic sites are identified as temporal pattern and spatial pattern, respectively. Also highlighted are the stage gauges used to determine the hydrologic gradient. Structures regulating discharges and releases of water are indicated. The direction of flow in the L-7, L-39 and L-40 canals is indicated by the black arrows.

could not be calculated through summation, it was estimated from conductivity ($\mu S\ cm^{-1}$) using linear regression ($r^2 = 0.98$; $y_0 = -14.6$; $a = 0.672$).

Numerical methods

The development of system-wide (i.e. Everglades peatland) transfer functions followed Birks (2010). The data set was comprised of 911 periphytometer samples, collected between 1994 and 2005, with corresponding measurements of TP, TKN, salinity, Ca^{2+}, Mg^{2+}, Na^{+}, K^{+}, SO_4^{2-}, Cl^{-} and HCO_3^{-}. Parameters were paired with periphytometers by averaging within the 6-to-8-week periphytometer deployment. The taxonomic data set was reduced to include taxa with five or more occurrences and relative abundances >1%. Taxonomic proportional data were arcsine-square-root (ASR)-transformed, and deployment average concentrations were log-transformed to reduce skewness and improve linearity (McCune & Grace, 2002). The samples represented all the major regions (WCA-1, WCA-2A, WCA-3A, WCA-3B and Everglades National Park), habitat types (oligotrophic, eutrophic, ombrotrophic, minerotrophic, short hydroperiod and long hydroperiod) and seasons (wet and dry). Water-quality summary statistics associated with the 911 periphytometer samples are presented in Table 1.

We performed the following preliminary analyses to determine the appropriateness of unimodal models for reconstructing water quality. First, a detrended correspondence analysis (DCA) was used to estimate gradient length (Birks, 2010). The first two axes of the DCA accounted for 8.3% of the cumulative species variance which is typical for large noisy data sets containing many zeros. A gradient length equal to 3.298 for ASR transformed species relative abundances with no down-weighting of rare taxa confirmed unimodality in the species data. Second, a canonical correspondence analysis (CCA) with ASR transformed species relative abundances with down-weighting of rare taxa was then used to explore relationships among and between taxa and the water-quality variables. Using significant regression coefficient t-values and variance inflation factors (VIF) >20 as redundancy cut-off values, the water-quality data set was reduced to the predictor variables log(salinity), log(TP) and log(TKN). Analyses were carried out using CANOCO version 4.5 (Microcomputer Power, Ithaca, NY, U.S.A.).

Table 1 System-wide summary statistics of water-quality parameters that corresponded with the 911 periphytometer samples used to develop transfer functions

Parameter	Mean ± SD	Q1	Median	Q3	Range	*n*
Total phosphorus	0.022 ± 0.029	0.008	0.013	0.024	0.004–0.377	911
Total Kjeldahl nitrogen	1.75 ± 0.78	1.1	1.7	2.2	0.41–8.15	911
Salinity	468 ± 263	269	443	678	31–1666	911
Dissolved calcium	56 ± 26	42	62	75	2.7–148	911
Dissolved magnesium	15 ± 11	4.8	14	24	1.0–53	911
Dissolved sodium	58 ± 43	18	55	91	1.9–350	911
Dissolved potassium	4.4 ± 2.9	1.7	4.6	6.9	<0.1–11	911
Dissolved bicarbonate	214 + 105	147	230	285	5.1–582	911
Dissolved chloride	85 ± 64	28	79	130	1.2–605	911
Dissolved sulphate	24 ± 25	1.6	28	79	0.1–122	911
Dissolved silica	12 ± 7.4	5.1	11	18	0.3–46	911
Nitrate + nitrite	0.010 ± 0.019	0.004	0.005	0.009	0.002–0.290	911
Dissolved iron	0.051 ± 0.088	0.009	0.014	0.053	0.003–0.872	911
pH	7.19 ± 0.44	7.07	7.27	7.44	5.54–8.51	852
Conductivity	630 ± 365	331	594	914	49.7–2647	849
Dissolved oxygen	3.2 ± 2.0	1.6	2.7	4.3	0.1–11.7	852
Dissolved organic carbon	27 ± 12	19	28	37	3.6–86	909

All units are mg L^{-1} except for conductivity (μS cm^{-1}) and pH.

With the unimodal model approach confirmed, we performed another CCA with forward selection applying Monte Carlo testing (999 unrestricted permutations; $P \leq 0.01$) and biplot scaling to determine whether periphyton data were significantly distributed along log(salinity), log(TP) and log(TKN) gradients (ter Braak, 1986). The explanatory power of each water-quality variable was then assessed using partial canonical correspondence analysis (ter Braak, 1988). Individual taxa optima and tolerances were derived from weighted average (WA) regression using C2, version 1.4 Beta (Steve Juggins, Newcastle, U.K.).

Periphyton-based transfer functions for log(salinity), log(TP) and log(TKN) were constructed using weighted averaging partial least squares (WA-PLS) regression with leave-one-out cross-validation (ter Braak & Juggins, 1993; Birks, 2010). A subset of 456 periphytometer samples were randomly selected from the pool of 911 to use as the model training set. Comparison of the relationship between observed and periphyton-inferred concentrations for the training set only (r^2_{apparent}), between observed and cross-validation estimates ($r^2_{\text{jackknife}}$) and residuals were used to assess the predictive ability of each transfer function. Model selection was based on (i) the root mean square error of the training set (RMSE); (ii) the component with the lowest root mean square error of prediction (RMSEP) derived by cross-validation, lowest mean bias and greatest maximum bias; (iii) RMSEP reductions >5%; and (iv) significant *t*-test derived from repeated randomisation (Birks, 2010). The remaining 455 samples served as a calibration data set to further validate the predictive ability of each transfer function. Periphyton-inferred concentrations for the calibration data set were derived using the transfer function and 10-fold cross-validation. Model performance was assessed by comparing observed with periphyton-inferred concentrations. Transfer functions were developed using C2, version 1.4 Beta (Steve Juggins, Newcastle, U.K.).

Case study

To investigate the utility of periphyton-based transfer functions as tools to assess biological responses to management actions, we compared reconstructed (inferred) salinity and TP spatial and temporal patterns in WCA-1 with measured concentrations and management practices. WCA-1 is a 572-km^2 ombrotrophic subtropical peatland (latitude 26°30′N; longitude 80°20′W) impounded by 93 km of perimeter canals and levees (Fig. 1). This impoundment was chosen because it is impacted by multiple water-quality stressors and has experienced major changes in water management. The contrast between perimeter canals and interior marsh concentrations is considerable, with canals having elevated mineral and P concentrations relative to the predominantly rain-driven marsh interior (Newman & Hagerthey, 2011). Canal-water intrusions are evident by patterns in water quality (Harwell *et al.*, 2008; McCormick, Harvey & Crawford, 2011; Newman & Hagerthey, 2011), soil with elevated TP and Ca contents and monotypic stands of

Typha domingensis Pers. (Newman & Hagerthey, 2011) and shifts in periphyton species composition (Gaiser *et al.*, 2011).

Periphytometers have been used since 1996 to assess biological dynamics along two transects in south-western WCA-1 (Fig. 1) that span both the TP and salinity gradients (McCormick *et al.*, 2011; Newman & Hagerthey, 2011). Salinity and TP were reconstructed by applying the system-wide WA-PLS transfer functions to 409 periphytometer samples collected between 1996 and 2009. Sample-specific standard errors of reconstructed water quality were obtained with bootstrap (1000 cycles) cross-validation (Birks *et al.*, 1990). Reconstructions were derived using C2, version 1.4 Beta (Steve Juggins, Newcastle, U.K.).

To further simplify the communicative ability of the biological assessment, a tricolour code was developed that relates periphyton-inferred concentrations of TP and salinity to concentration benchmarks that concern Everglades resource managers and stakeholders. The benchmark for TP was 0.010 mg L^{-1}, which reflects the numeric nutrient criterion set forth in subsection 62–302.540 of the Florida Administrative Code (F.A.C.). Although the criterion represents a long-term geometric mean of 0.010 mg TP/L, it provides the context to evaluate and report biological responses to management actions, restoration efforts or natural variation. Inferred concentrations ≤0.010 mg TP/L were coded green, signifying a periphyton assemblage comprising natural and desirable Everglades flora. Inferred concentrations between 0.011 and 0.019 mg TP/L were coded yellow, indicating an assemblage not entirely comprising natural flora, and may include indicators of eutrophication. Red coded values represented inferred concentrations >0.020 mg TP/L, reflecting an assemblage probably comprising eutrophic taxa not indicative of natural flora. Although numeric nutrient criterion for salinity has not been established for the Everglades, there is a growing desire to protect the remaining soft-water ecosystems and concern regarding the discharge of hard water from canals into the marsh. Best professional judgement was used to establish thresholds of 100 and 350 mg L^{-1} that distinguished soft-water assemblages from hard-water assemblages (Gaiser *et al.*, 2011; Hagerthey *et al.*, 2011). Soft-water assemblages (inferred salinities < 100 mg L^{-1}) were coded green, transitional assemblages (100–350 mg L^{-1}) were coded yellow, and hard-water assemblages (>350 mg L^{-1}) were coded red.

To assess biological responses to water management practices, we characterised the daily contribution of structural operations to the hydrologic budget and nutrient loading to WCA-1. Since 1995, structure operations has been guided by the U.S. Army Corp of Engineers derived WCA-1 Water Regulation Schedule, which defines daily marsh stage targets and recession rates for the benefit of wildlife as well as operational guidance for flood control and water supply (Brandt, 2006). However, significant changes in operations and water management have occurred over the past 15 years as infrastructure has been modified to reduce P loads. Major climate events (droughts and hurricanes) have also influenced operations. We chronologically reduced the study period to four operational and three climatic periods (Table 2). The operational periods (A, C, F and G) captured major infrastructure changes, whereas the climate periods (B, D and E) captured the 2000/2001 drought, the 2003/2004 drought and the 2004 hurricane season. These events triggered temporary deviations from the regulation schedule that affected operations for an extended period of time (>3 months) in 2001 and 2005 (Brandt, 2006). Water management practices were summarised as the mean daily values for each operational period (Table 3) and cumulative daily load (Fig. 2). Hydrologic inflows, hydrologic outflows, salinity and P loads, salinity and P flow-weighted means, and water stage and depth were calculated using data obtained from the South Florida Water Management District's DBHYDRO database (http://www.sfwmd.gov/DBHYDRO; see Table 3 for details).

Biological responses to management were assessed by comparing site-specific periphyton-inferred salinity and TP concentrations across operational periods A, C, F and G using one-way analysis of variance (ANOVA) and Tukey's HSD tests with $\alpha = 0.05$ for pairwise comparisons. Periods B, D and E were not included in the statistical analysis owing to the small sample size within each period. Statistical procedures were completed using SAS-JMP, version 8.0.1 (SAS Institute, Cary, NC, U.S.A.).

Results

The Everglades peatland is comprised of a diverse algal assemblage. A total of 1430 algal taxa were identified from the 3800 periphytometer samples collected over the 15-year monitoring period. The data set was represented by 582 desmids, 326 diatoms, 94 coccoid cyanobacteria, 65 filamentous cyanobacteria, 43 heterocystic cyanobacteria, 134 non-motile chlorophytes, 26 filamentous chlorophytes, 22 flagellated chlorophytes, 23 chrysomonads, 11 euglenoids, three cryptomonads, 17 dinoflagellates, 30 heterokonts and one rhodophyte. Many of the taxa, however, were numerically and proportionally rare, with 610 taxa

Table 2 The seven periods of Water Conservation Area 1 water management between 1996 and 2010

		L-7 West structures				L-39	L-40 East structures				
Period	Start date	S5A	G251	G301	G310	S6	S5AS	G300	S362	ACME	Operations
A	January 1996	X	X			X	X			X	S5A and S6 pump stations deliver mostly untreated water to WCA-1 Regulation schedule deviation (January–December 1998) to hold more water in WCA-1 in response to regionally high water levels
B	October 2000		X	X	X	X		X		X	Minimal deliveries made to WCA-1 owing to a system-wide drought, which resulted in a regulation schedule deviation to meet water supply demands
C	July 2001		X	X	X			X		X	Major infrastructure changes, S5A discharges were diverted to storm water treatment area (STA)-1 West and delivered to L-7 canal S6 discharges were completely and permanently diverted from WCA-1
D	October 2003		X	X	X			X		X	System-wide drought
E	July 2004		X	X	X			X		X	Hurricane Frances (Cat 2) impact
F	October 2004		X	X	X			X	X	X	Storm water treatment area-1 East began water deliveries to L-40 canal Regulation schedule deviation (April 2005–July 2006) to reduce P loading
G	September 2006		X	X	X			X	X	X	Management with all infrastructure operational and no regulation schedule deviations

The periods are differentiated by the operation of inflow structures, water treatment and climatic events.
Pump capacities – S5A = 112 $m^3\ s^{-1}$; G251 = 12.6 $m^3\ s^{-1}$; S6 = 82 $m^3\ s^{-1}$; G310 = 85 $m^3\ s^{-1}$; S362 = 118 $m^3\ s^{-1}$; ACME 1 and 2; 9.8 $m^3\ s^{-1}$.
Spillway capacities – S5AS = 56 $m^3\ s^{-1}$; G301 = 28 $m^3\ s^{-1}$; G300 = 28 $m^{3-1}s^{-1}$.

having counts fewer than five and 1048 taxa having relative abundances <1%.

Transfer functions

The 911 periphytometer data set used to develop the transfer functions was comprised of 319 taxa having relative abundances ≥1 and ≥5 occurrences. The composition consisted of 68 desmids, 103 diatoms, 45 coccoid cyanobacteria, 30 filamentous cyanobacteria, 12 heterocystic cyanobacteria, 28 non-motile chlorophytes, 11 filamentous chlorophytes, four flagellated chlorophytes, nine chrysomonads, three euglenoids, one cryptomonad, two dinoflagellates and three heterokonts.

The first two axes of the CCA with ASR transformations and down-weighting of rare species accounted for 7.9% of the cumulative species variance in the weighted averages of algal taxa; the eigenvalues were 0.188 for axis 1 and 0.102 for axis 2 (Fig. 3). Patterns were similar between the constrained and unconstrained ordinations. A large proportion of the species–environment relationships were also captured by the first two axes (axis 1 = 60.8%; axis 2 = 32.9%). There was a strong relationship between species and the three environmental variables with species–environment correlation for axis 1 = 0.90 and axis 2 = 0.77 (Fig. 3a), indicating that taxa were distributed along an environmental gradient. Salinity, TP and TKN made significant and independent contributions to aid in interpreting the underlying variation in the algal compositions (Fig. 3b).

Optima and tolerances for salinity, TKN and TP for the 319 spanned the freshwater salinity and eutrophication continuum (see Table S1). Species weights ranged from 0.04 to 288 with 43 and 71% having weights >5 and 1, respectively. Salinity optima ranged from 39 to 867 mg L^{-1}, TKN from 0.61 to 3.05 mg L^{-1} and TP from 0.004 to 0.150 mg L^{-1}. An exponential curve best described the overall relationship between salinity and TP optima (TP = $7.16\exp^{0.0022\text{salinity}}$; $r^2 = 0.52$); however, patterns differed among the taxonomic groups with no one group adequately spanning the observed salinity and TP ranges (Fig. 4). Desmids were generally restricted to oligotrophic soft waters, whereas diatoms, cyanobacteria and other chlorophytes were less prevalent (Fig. 4). The majority of taxa (306) had TP optima <0.050 mg L^{-1}. Sixty-nine taxa had optima ≤0.010 mg TP/L, with desmids accounting for 62% of the taxa. *Mastogloia smithii*, *Encyonema evergladianum*, *Gomphonema vibrio*, *Nitzschia*

Table 3 WCA-1 hydrology and stage summaries for operational and climatic periods between the L-7 canal and marsh

Period	A	B	C	D	E	F	G
Start date	January 1996	October 2000	July 2001	October 2003	July 2004	October 2004	September 2006
End date	October 2000	July 2001	October 2003	July 2004	October 2004	September 2006	December 2009
WCA-1 Hydrologic Budget ($m^3 \times 10^5$ day^{-1})							
Inflow ($F_{6,\ 3316}$ = 64.1; P < 0.0001)	21.7 ± 33.6^{b}	2.5 ± 5.3^{d}	17.5 ± 20.3^{b}	5.3 ± 8.6^{cd}	57.5 ± 44.0^{a}	9.5 ± 16.7^{c}	9.2 ± 18.5^{d}
S6 ($F_{1,\ 1945}$ = 156.9; P < 0.0001)	9.5 ± 14.7	0.8 ± 3.4					
West ($F_{6,\ 4933}$ = 301.9; P < 0.0001)*	10.6 ± 16.9^{b}	0.9 ± 3.0^{f}	16.2 ± 18.8^{c}	4.9 ± 8.4^{d}	48.9 ± 31.8^{a}	6.0 ± 9.5^{d}	5.1 ± 12.3^{e}
East ($F_{6,\ 4933}$ = 50.1; P < 0.0001)*	1.6 ± 4.4^{d}	1.1 ± 1.9^{d}	1.3 ± 3.0^{d}	0.4 ± 1.3^{e}	8.6 ± 14.7^{c}	3.5 ± 8.3^{a}	4.1 ± 7.6^{b}
Outflows ($F_{6,\ 4294}$ = 40.5; P < 0.0001)*	18.8 ± 33.8^{b}	4.6 ± 6.5^{bc}	15.6 ± 18.5^{a}	5.8 ± 8.1^{b}	49.2 ± 50.4^{ab}	7.2 ± 17.4^{cd}	9.8 ± 19.1^{d}
Precipitation ($F_{6,\ 4294}$ = 17.0; P < 0.0001)	23.1 ± 63.0^{bc}	11.1 ± 34.8^{e}	20.8 ± 46.3^{b}	10.9 ± 28.8^{de}	40.8 ± 67.5^{a}	18.4 ± 39.6^{cd}	19.9 ± 52.7^{cd}
ET ($F_{6,\ 4294}$ = 1.9; P = 0.0814)	21.2 ± 7.7	21.8 ± 6.8	20.4 ± 69.7	21.3 ± 7.2	19.8 ± 5.7	20.5 ± 7.1	20.3 ± 6.3
Salinity Load (Mt day^{-1})							
S6 ($F_{1,\ 1945}$ = 157.2; P < 0.0001)	768 ± 38	53 ± 83					
West ($F_{6,\ 4933}$ = 301.1; P < 0.0001)	802 ± 27^{b}	71 ± 68^{f}	1221 ± 39^{c}	348 ± 70^{d}	3459 ± 127^{a}	456 ± 43^{d}	353 ± 30^{e}
East ($F_{6,\ 4933}$ = 52.2; P < 0.0001)	77 ± 253^{c}	57 ± 100^{c}	65 ± 180^{c}	24 ± 86^{d}	389 ± 635^{a}	219 ± 544^{b}	199 ± 360^{ab}
P Load (kg day^{-1})							
S6 ($F_{1,\ 1945}$ = 147.7; P < 0.0001)	108 ± 237	7 ± 36					
West ($F_{6,\ 4933}$ = 199.0; P < 0.0001)	131 ± 346^{b}	5 ± 19^{e}	83 ± 106^{b}	15 ± 27^{d}	631 ± 700^{a}	77 ± 150^{c}	22 ± 49^{d}
East ($F_{6,\ 4933}$ = 35.3; P < 0.0001)	21 ± 75^{cd}	8 ± 17^{d}	15 ± 44^{d}	4 ± 17^{e}	268 ± 517^{a}	44 ± 127^{b}	15 ± 38^{bc}
Salinity Flow-Weighted Mean (mg L^{-1})							
S6 ($F_{1,\ 1945}$ = 46.8; P < 0.0001)	779 ± 193	654 ± 90					
West ($F_{6,\ 3528}$ = 3.6; P = 0.0014)	758 ± 205^{b}	787 ± 54^{ab}	751 ± 153^{b}	729 ± 104^{ab}	737 ± 100^{ab}	783 ± 143^{a}	750 ± 121^{ab}
East ($F_{6,\ 1881}$ = 79.7; P < 0.0001)	449 ± 108^{d}	534 ± 87^{b}	465 ± 102^{cd}	529 ± 150^{bc}	492 ± 85^{bc}	610 ± 131^{a}	531 ± 138^{b}
TP Flow-Weighted Mean (μg L^{-1})							
S6 ($F_{1,\ 1945}$ = 237.7; P < 0.0001)	97 ± 79	50 ± 22					
West ($F_{6,\ 3528}$ = 142.2; P < 0.0001)	57 ± 61^{c}	61 ± 58^{bc}	58 ± 57^{b}	30 ± 5^{d}	110 ± 70^{a}	114 ± 46^{a}	52 ± 64^{b}
East (F = 517.7; P < 0.0001)	116 ± 24^{b}	74 ± 41^{c}	103 ± 39^{c}	93 ± 37^{cd}	217 ± 108^{a}	101 ± 50^{c}	35 ± 38^{e}
Stages (m) and Depth (cm)							
Marsh stage ($F_{6,\ 4954}$ = 98.0; P < 0.001)†	5.06 ± 0.14^{a}	4.91 ± 0.18^{d}	5.06 ± 0.11^{a}	4.98 ± 0.13^{c}	5.07 ± 0.14^{a}	4.96 ± 0.10^{c}	5.02 ± 0.14^{b}
Canal stage ($F_{6,\ 4929}$ = 100.4; P < 0.001)†	4.98 ± 0.23^{a}	4.66 ± 0.38^{d}	4.99 ± 0.21^{a}	4.77 ± 0.32^{c}	5.01 ± 0.14^{a}	4.90 ± 0.15^{b}	4.90 ± 0.28^{b}
Difference ($F_{6,\ 4929}$ = 96.7; P < 0.001)†	−0.08 ± 0.14^{b}	−0.25 ± 0.22^{e}	−0.08 ± 0.13^{b}	−0.21 ± 0.19^{d}	−0.06 ± 0.06^{ab}	−0.07 ± 0.09^{a}	−0.12 ± 0.15^{c}
Marsh depth ($F_{6,\ 4954}$ = 102.1; P < 0.001)	45 ± 14^{a}	30 ± 18^{d}	46 ± 11^{a}	38 ± 13^{c}	47 ± 14^{a}	36 ± 10^{c}	42 ± 14^{b}

Values were compared between periods A, C, F, and G with one-way ANOVA and Tukey's HSD tests. Periods with the same superscript are not significantly different at a significance level (α) of 0.05.

*Structures used to calculate west inflows were S5A, S5AS and G251 for periods A and B and G251, G301 and G310 for periods C–G. Structures for east inflows were ACME1 and ACME2 for period A, ACME1, ACME2 and G300 for periods B and C, ACME1, ACME2, G300 and S362 for periods D–F, and G300 and S362 for period G. Outflows were calculated using structures S10A, S10C, S10D, S10E, S39 and G94A–D.

†The 1–7 gauge (was used for the marsh stage and the S10-E headwater gauge for canal stage referenced to NGVD29. Difference was calculated as canal (S10-E)-marsh (1–7).

serpentiraphe and Schizothrix calcicola, taxa typically listed as indicators of oligotrophy, had TP optima greater than the 0.010 mg L^{-1}; thus, the presence of these taxa were not uncontestable indicators of the P benchmark.

For the three water-quality variables, the second WA-PLS component was the minimum adequate model required to infer concentrations from periphytometers (Table 4). For the 456 sample training set (Fig. 5a–c), measured versus periphyton-inferred concentrations were strongly related for salinity ($r^2_{jackknife}$ = 0.81; RMSEP = 0.15 mg L^{-1}) and TP ($r^2_{jackknife}$ = 0.70; RMSEP = 0.21 mg L^{-1}), but weakly related for TKN ($r^2_{jackknife}$ = 0.46; RMSEP = 0.15 mg L^{-1}). Average and maximum bias equalled −0.003 and 0.36 mg L^{-1} for log(salinity), 0.002 and 0.52 mg L^{-1} for log(TKN) and 0.004 and 0.67 mg L^{-1} for log(TP). Residual plots for each model indicated that there was an overall tendency for the models to overestimate concentrations at the low end of the gradient and underestimate at the high

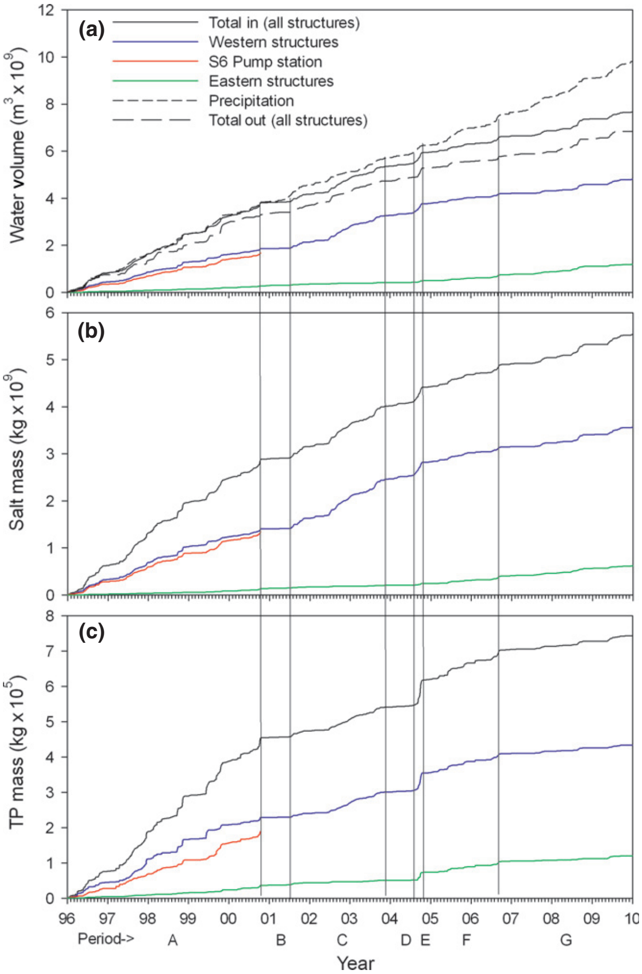

Fig. 2 Daily cumulative load of (a) hydrology, (b) salinity and (c) total phosphorus into WCA-1 from all structures (total), the S6 pump station, the combined western structures and the combined eastern structures. Management periods (a–e) are demarked by the solid vertical lines.

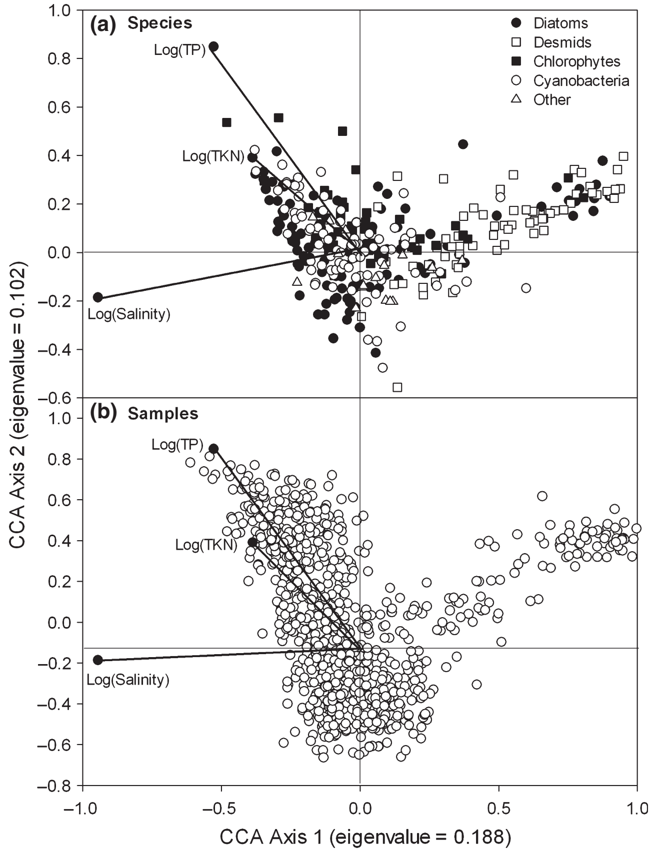

Fig. 3 CCA biplot of (a) periphyton species and the environmental variables log(salinity), log TKN and log total phosphorus (TP) and (b) periphytometer samples and environmental variables. The ordination was derived from 911 periphytometer samples deployed between 1996 and 2005 and from the square-root-transformed relative abundances of 319 taxa that had >5 occurrences and a relative abundance >1%. Rare taxa were down-weighted. For axis 1 and axis 2, the eigenvalues were 0.188 and 0.102 and accounted for 5.1 and 2.8% of the variance, respectively.

end of the gradient (Fig. 5d–f). Model validations were assessed by comparing reconstructed concentrations obtained for the 455 sample validation data set utilising the second component WA-PLS transfer function with observed concentrations (Fig. 6). The log-transformed salinity (r^2 = 0.78) and TP (r^2 = 0.65) transfer functions provided a good estimate of observed concentrations, whereas the TKN transfer function did not (r^2 = 0.38).

Biological assessment of water conservation area 1

Since inferred salinity and TP were derived from biological data (periphyton), they served as metrics to the biological status of WCA-1; that is, spatial and temporal trends and colour coding of inferred concentrations were analogues to the trends and status of periphyton. Tricolour coding the inferred concentrations simplified the interpretation and presentation of biological status. By converting a large complex species-abundance matrix to 'common' metrics, biological responses and trends are more readily apparent to individuals not trained in taxonomy or multivariate statistical methods commonly used to summarise biological patterns. In addition, the use of multiple transfer functions allowed for the simultaneous assessment of multiple stressors rather than focusing on a single variable. Thus, a more holistic understanding of the factors affecting biological patterns was attained.

Reconstruction of log-transformed salinity and TP concentrations varied among the 10 monitoring sites with some sites exhibiting strong temporal patterns (Figs. 7 & 8). Given the poor performance of the TKN transfer function, TKN reconstructions were not made. The ranges

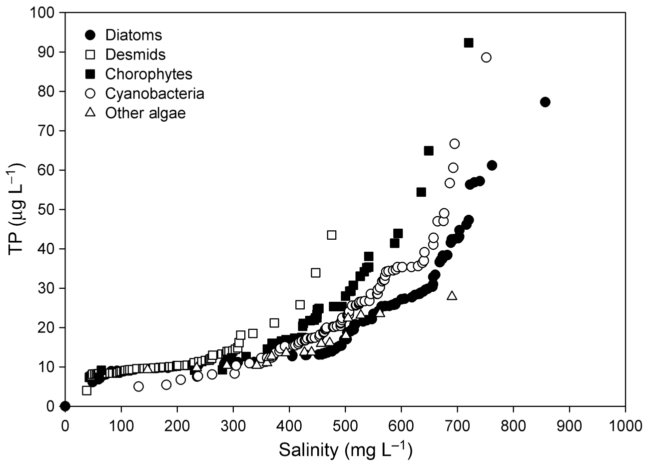

Fig. 4 Paired salinity (mg L^{-1}) and TP (µg L^{-1}) optima for individual taxa grouped according to taxonomic classification. Solid lines indicate the boundaries for tricoloured categories and include the 10 µg P/L numeric criterion.

of sample-specific inferred standard errors were 0.023–0.13 mg L^{-1} for salinity and 0.02–0.09 mg L^{-1} for TP. Among sites, inferred salinity and TP captured the well-established spatial gradients (Figs 7 and 8), which were visually apparent as the transition from regnant red values from the canal (X1 and Z1) to green in the marsh interior (MESO). Within-site temporal patterns were more apparent for salinity than TP. Inferred salinity remained invariant for sites close to the canal and marsh interior, whereas concentrations for intermediate sites approximated a unimodal time-series with the effect being greater for sites further from the canal (Fig. 7). Inferred concentrations typically mirrored measured concentrations with the exception of period F when inferred salinity lagged measured declines (Fig. 7). The tricolour coding system made clear the magnitude of within-site transitions. Site-specific inferred TP remained fairly constant over time, which was visually represented by the propensity of the colour code to remain invariant (Fig. 8). Within-site inferred log-transformed salinity and TP generally differed significantly among the operational periods for sites distant from the canal and marsh interior (see Table S2). Contrary to expectations, the lowest inferred TP for individual sites tended to occur in periods C and F when inferred salinities were greatest (Figs 7 and 8).

Discussion

Model development

The traditional application of transfer functions is to infer historic environmental conditions (Smol & Stoermer, 2010) from species-response models (Birks, 2010). This application requires a robust fossil record to infer environmental conditions using modern taxonomic assemblages. Diatoms are ideal for this application because their siliceous cell walls are well preserved in aquatic environments and taxonomically diagnostic (Smol & Stoermer, 2010), whereas soft algae are not well preserved. It is not surprising, therefore, that the literature abounds with diatom-based transfer functions (Smol & Stoermer, 2010), but is sparse with algal-based indices (c.f., King *et al.*, 2000; Munn *et al.*, 2002; DeNicola *et al.*, 2004). Whilst diatom-based indices are the preferred means to reconstruct past environmental conditions, both can be used to

Table 4 Performance of weighted averaging partial least squares components with leave-one-out cross-validation for the water-quality variables log-transformed salinity, total Kjeldahl nitrogen (TKN) and TP

Environmental variable (mg L^{-1})	WA-PLS component	$r^2_{apparent}$	$r^2_{jackknife}$	RMSE mg L^{-1}	RMSEP mg L^{-1}	% change	*P*-value
Log(salinity)	1	0.77	0.75	0.17	0.18	–	–
	2	0.87	0.81	0.13	0.15	13.2	0.001
	3	0.89	0.82	0.12	0.15	1.3	0.182
	4	0.91	0.81	0.10	0.16	−3.1	0.975
	5	0.92	0.79	0.10	0.16	−3.7	0.998
Log(TKN)	1	0.44	0.37	0.15	0.16	–	–
	2	0.60	0.46	0.12	0.15	6.9	0.001
	3	0.69	0.46	0.11	0.15	0.3	0.424
	4	0.74	0.43	0.10	0.15	−5.0	1.000
	5	0.77	0.41	0.09	0.16	−3.7	0.998
Log(TP)	1	0.70	0.66	0.21	0.22	–	–
	2	0.77	0.70	0.18	0.21	7.5	0.001
	3	0.84	0.70	0.15	0.21	−0.3	0.571
	4	0.87	0.68	0.14	0.21	−4.0	0.999
	5	0.88	0.67	0.13	0.22	−2.6	0.997

In each case, component 2 yielded the minimum adequate model required to inferred concentrations throughout the Everglades.

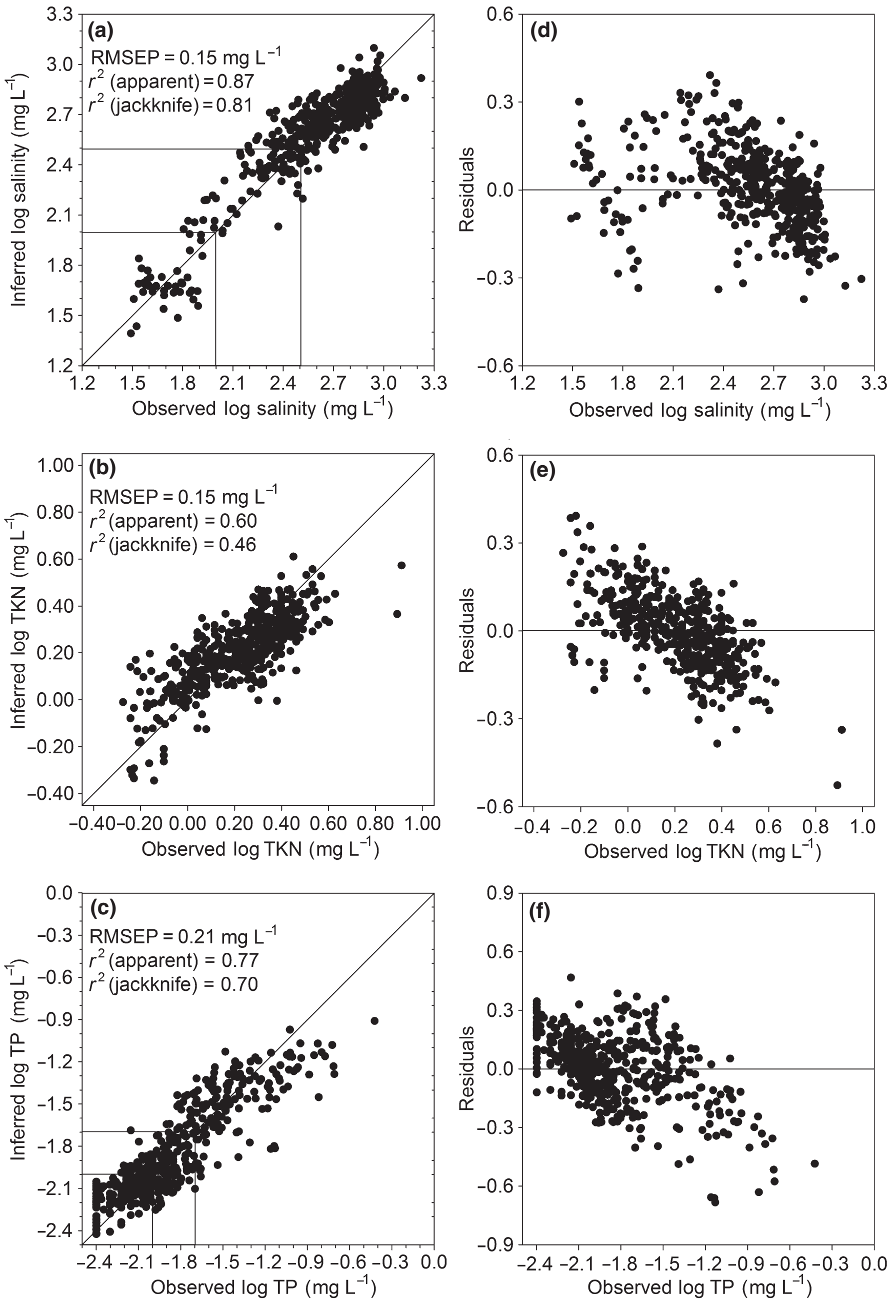

Fig. 5 Relationship between measured and periphyton-predicted log (a) salinity, (b) total Kjeldahl nitrogen (TKN) and (c) total phosphorus (TP) derived from the second components of weighted average partial least squares regression (r^2_{apparent}) and leave-one-out cross-validation ($r^2_{\text{jackknife}}$) using 456 periphytometer sample training set. Also shown are the measured versus model residuals for (d) salinity, (e) TKN and (f) TP. Solid lines indicate the boundaries for tricoloured categories that include the 0.010 mg P/L numeric criterion.

assess current trends in or responses to restoration or management actions using modern-day assemblages. By broadening the taxonomic composition to include soft algae (e.g. chlorophytes, cyanobacteria and chrysophytes), the predictive ability of periphyton-based transfer functions should be better than diatom-based models owing to the increase in the number of species-response models (King *et al.*, 2000). Indeed, the predictive ability of diatom-based transfer functions developed for the Everglades was not as good as that of the periphyton-based transfer functions (Table 5; S. Hagerthey, unpubl. data).

Periphyton type is an important factor in the development and interpretation of periphyton-based transfer functions. Periphyton types vary greatly, from thick cohesive mats to amorphous, gelatinous clouds (Hagerthey *et al.*, 2011), affected by the substratum on which they

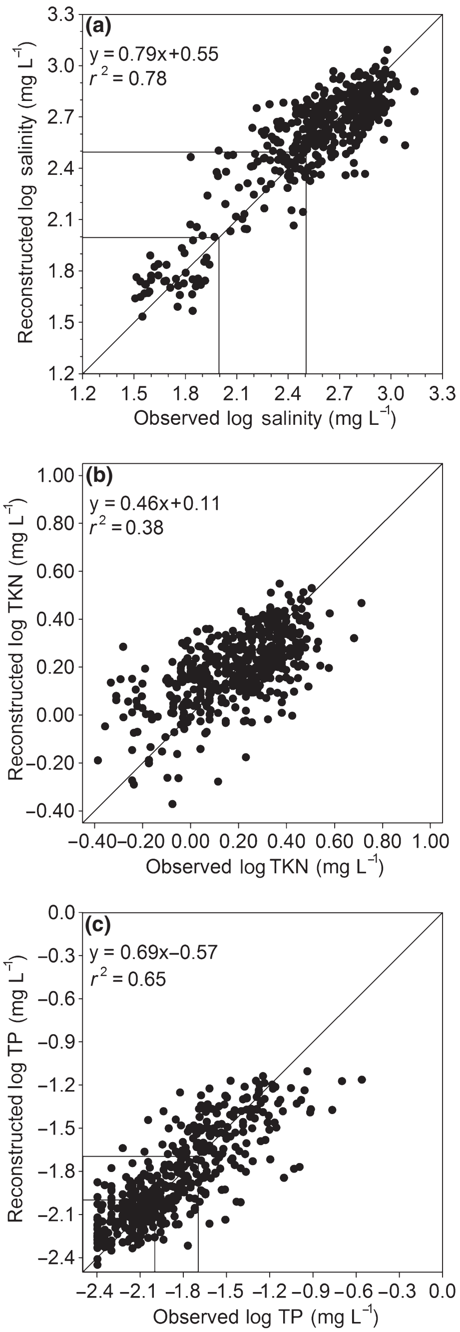

Fig. 6 Validation of periphyton-based transfer function performance for log (a) salinity, (b) total Kjeldahl nitrogen and (c) total phosphorus derived from comparing measured and reconstructed concentrations for an independent 455 periphytometer sample data set.

occur (Stevenson, 1996). This diversity can either aid or hinder periphyton-based indices. To date, periphyton-based transfer functions have been derived mostly from algae collected from solid substrata, such as rocks (*epilithon*; King *et al*., 2000; Munn *et al*., 2002; DeNicola *et al*., 2004), plants (*epiphyton*; Wachnicka *et al*., 2010) or artificial substrata (periphytometer, *this study*), with some attempts for cohesive mats and sediments (Munn *et al*., 2002; Wachnicka *et al*., 2010). Cohesive mats and sediment assemblages are, however, not ideal for developing transfer functions because individual samples often lack a relationship with a single water sample (Fritz, Juggins & Battarbee, 1993; but see Reed, 1998). The lack of a relationship can result from the accumulation and preservation of non-viable cells (Reed, 1998), the influence of other nutrient sources (Burkholder, 1996; Hagerthey & Kerfoot, 1998) or internal biogeochemical cycling (Hagerthey *et al*., 2011). Models based on solid substrata, whether natural or artificial, not affected by these factors to the same extent, are more likely to be regulated by variations in surface water chemistry and, thus, have a lower prediction error. Our use of artificial substrata (glass microscope slides) offered the advantages of standardising substrata across habitat types, minimising the effect of integrating taxa across seasons and improving the relationship between single measurements of water quality with assemblage composition (Aloi, 1990; Lane, Taffs & Corfield, 2003). The use of artificial substrata is not without criticism (Aloi, 1990), principally that assemblages on artificial substrata may not accurately reflect natural assemblages (Barbiero, 2000; Lane *et al*., 2003). However, for water-quality monitoring, artificial substrata are more advantageous because the replicability is greater and variability is lower (Barbiero, 2000; Lane *et al*., 2003).

Model assessment and performance

The periphyton-based transfer functions developed here fulfilled the core criteria requirements for biological indicators (Marchant *et al*., 2006). First, responses to environmental changes were rapid and predictable, indicating high degrees of sensitivity to ecosystem stressors, and thus were effective early warning indicators. Second, evaluation of salinity and TP transfer functions in combination provided valid causes of change and existence of degradation. Third, the conversion of complex taxonomic composition information to water-quality concentrations provided easily interpretable outputs related to management goals. A weakness of the models was that they were not very cost-effective as time and considerable expertise were required to perform the high-resolution taxonomic analyses; however, the benefit was an accurate and precise, therefore defensible, biological indicator.

The predictive abilities of our models were comparable to attached algal transfer functions developed for other aquatic ecosystems (Table 5). Unlike functions developed to reconstruct palaeoenvironments from fossil assemblage, where the poor preservation of other major algal groups

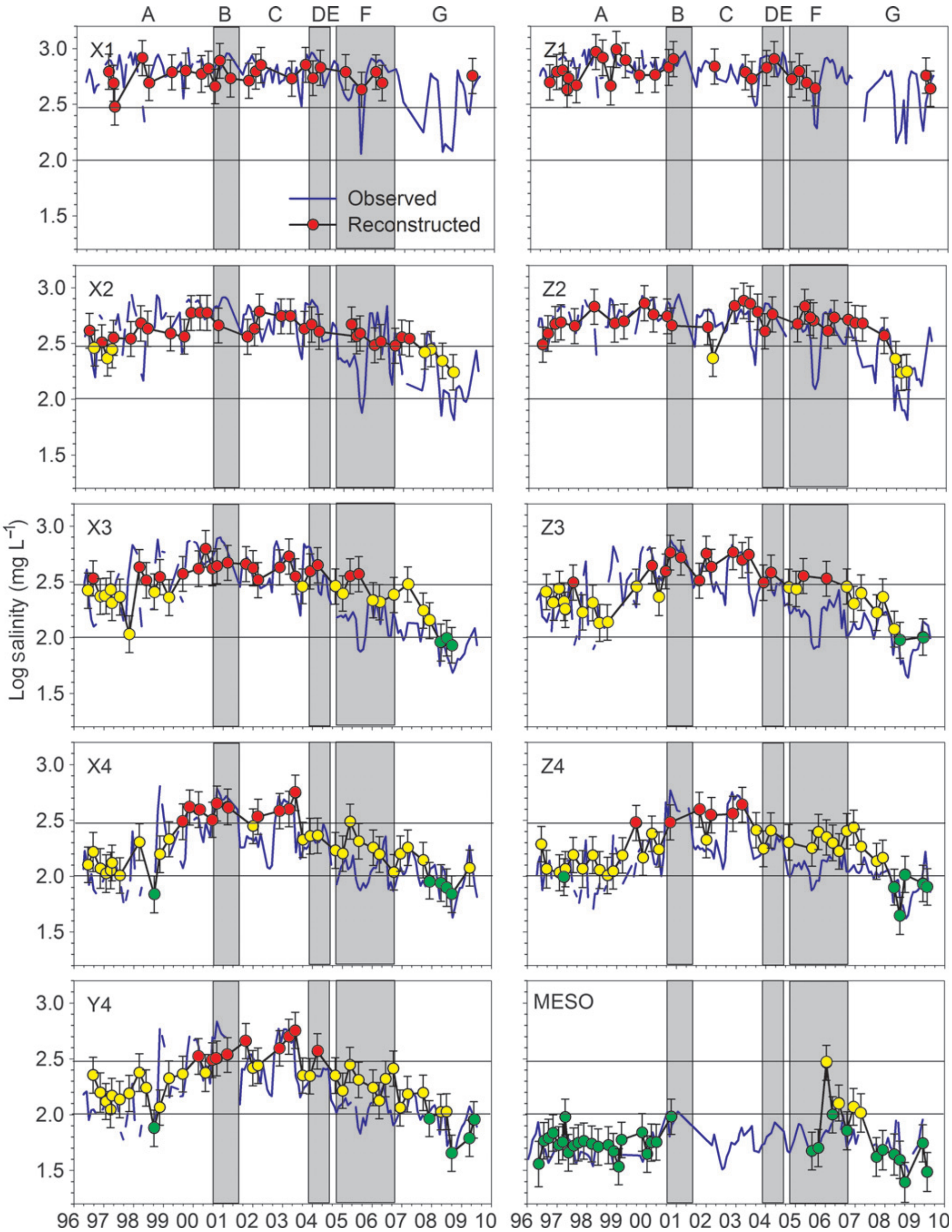

Fig. 7 Time-series of measured (solid line) and periphyton-based weighted averaging partial least square predictions (symbols) of log salinity for monitoring sites extending from the canal into the WCA-1 marsh interior. Error bars are the bootstrap-estimated sample-specific standard errors for inferred concentration. Symbols are tricolour-coded to indicate biologically inferred salinity below 100 mg L^{-1} (green), between 100 and 350 mg L^{-1} (yellow) and above 350 mg L^{-1} (red). Management periods (a–e) are demarked by the solid vertical lines.

(e.g. chlorophytes and cyanobacteria) reduces model performance (Stevenson, Pan & Van Dam, 2010), the inclusion of a more diverse species assemblage improved the performance of periphyton-based functions. In the case of the Everglades, predictability was improved by including 68 salinity-sensitive desmid taxa, 43 other chlorophytes and 87 cyanobacteria in the model, relative to models based solely on the diatom assemblage (Table 5).

For salinity, the predictive ability of the Everglades model ($r^2_{\text{jackknife}}$ = 0.81) was equivalent to that of models spanning a large salinity continuum (approximately 100 000 mg L^{-1}) (Table 5). Within the freshwater continuum (<1500 mg L^{-1}), the predictive ability of our model was similar to that of periphyton-based transfer functions (Munn *et al.*, 2002) but were better than that of the diatom-based functions (Tibby & Reid, 2004; Reid, 2005) and

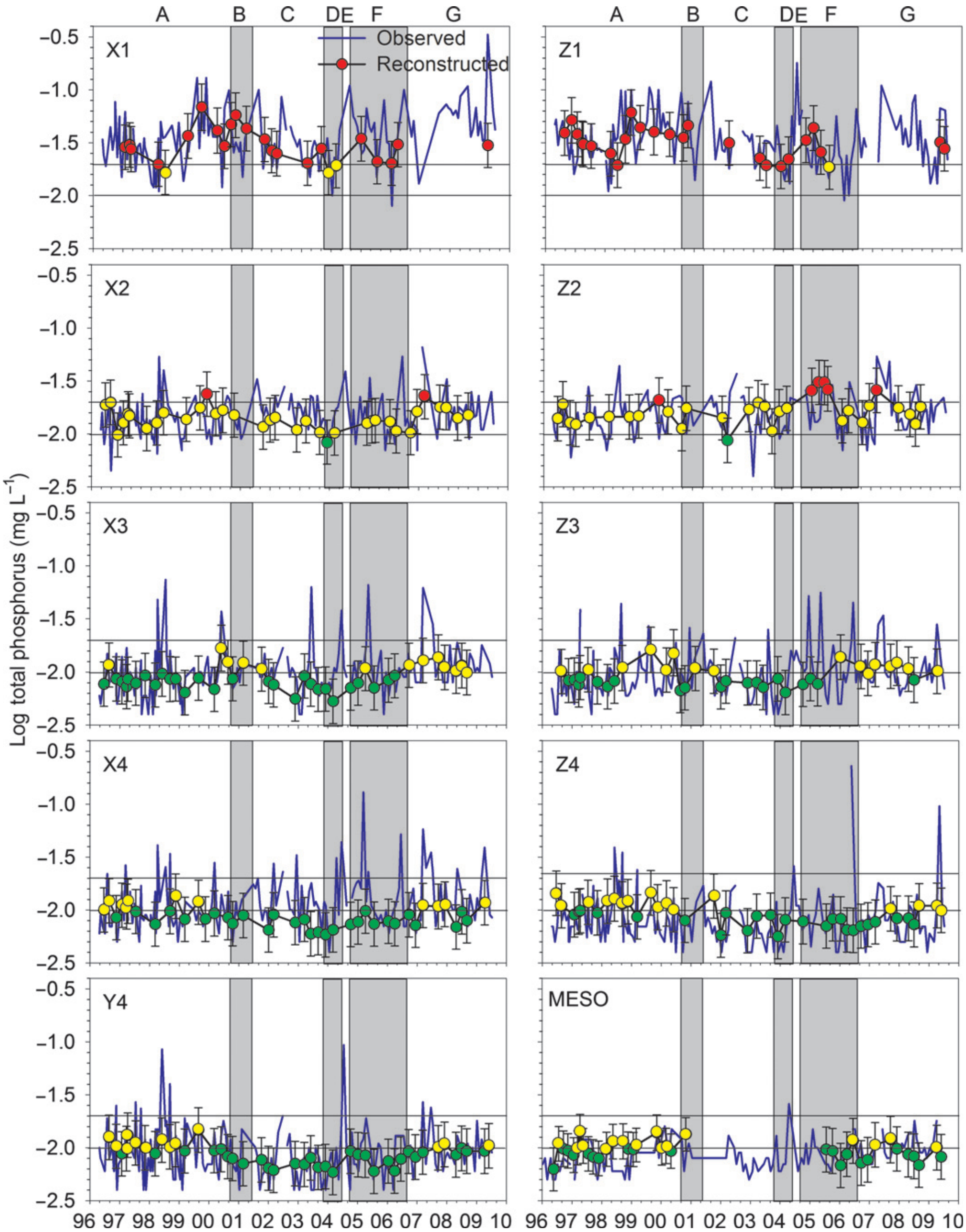

Fig. 8 Time-series of measured (solid line) and periphyton-based weighted averaging partial least square predictions (symbols) of log total phosphorus (TP) for monitoring sites extending from the canal into the WCA-1 marsh interior. Error bars are the bootstrap-estimated sample-specific standard errors for inferred concentration. Symbols are tricolour-coded to indicate biologically inferred TP below the 0.010 mg L^{-1} numeric criterion (green), between 0.010 and 0.020 mg L^{-1} (yellow) and above 0.020 mg L^{-1} (red). Management periods (a–e) are demarked by the solid vertical lines.

periphyton-based function that span short (<100 μS cm^{-1}) gradients (King *et al.*, 2000) (Table 5).

The predictive ability of the TP model for the Everglades peatland ($r^2_{\text{jackknife}}$ = 0.70) was better than that of other periphyton models and equivalent to that of two diatom-based functions (Table 5). The TP model showed evidence of the 'edge effect' (Birks, 1998), tending to overestimate below 0.006 mg TP/L and underestimate above 0.060 mg TP/L (Fig. 5f). The overestimation was likely due to the factitious truncation of the TP gradient associated with the minimum detection limits for TP (0.004 mg L^{-1}). Underestimation is commonly observed in diatom-TP models (Tibby, 2004; Ponader *et al.*, 2008) and presumably results from high concentrations not

Table 5 Comparison of salinity (conductivity), phosphorus and nitrogen transfer functions derived from using periphyton or benthic diatoms

	Range	Algal assemblage	Model type	r^2 Apparent	r^2 Cross-validation	References
Salinity or conductivity						
Lakes – U.K.	28–124 μs cm^{-1}	Periphyton	WA	0.64–0.65	0.47–0.50	King *et al.* (2000)
Lakes – Africa	40–99 060 μs cm^{-1}	Diatoms	WA	0.87–0.92	0.80–0.81	Gasse *et al.* (1995)
Lakes – Australia	40–156 000 mg L^{-1}	Diatoms	WA	0.95–0.97	0.88–0.89	Taukulis & John (2009)
Lakes – Greenland	24–4072 μs cm^{-1}	Diatoms	WA and WA-PLS	0.88–0.96	0.79–0.88	Ryves *et al.* (2002)
Lakes – New Zealand	20–1115 μs cm^{-1}	Diatoms	WA		0.53–0.79	Reid, 2005
Streams – U.S.A.	61–644 μs cm^{-1}	Periphyton	WA-PLS	0.91	0.83	Munn *et al.* (2002)
Rivers – Australia	53–1065 μs cm^{-1}	Diatoms	WA and WA-PLS		0.59–0.69	Tibby & Reid (2004)
Wetlands – Australia	328–133 795 μs cm^{-1}	Diatoms	WA and WA-PLS		0.82–0.89	Tibby *et al.* (2007)
Peatland – U.S.A.	31–1666 mg L^{-1}	Periphyton	WA-PLS	0.87	0.81	This Study
Peatland – U.S.A.	31–1666 mg L^{-1}	Diatoms	WA-PLS	0.73	0.71	S. Hagerthey, unpubl. data
Phosphorus						
Lakes – U.K.	0.001–0.049 mg L^{-1}	Periphyton	WA	0.60–0.64	0.45–0.47	King *et al.* (2000)
Lakes – Ireland	0.004–0.068 mg L^{-1}	Periphyton	WA	0.61–0.68		DeNicola *et al.* (2004)
Lakes – New Zealand	0.002–0.171 mg L^{-1}	Diatoms	WA		0.31–0.76	Reid (2005)
Reservoirs – Australia	0.007–0.451 mg L^{-1}	Diatoms	WA-PLS	0.74–0.94	0.52–0.74	Tibby (2004)
Coastal Zones – U.S.A.	0.006–0.139 mg L^{-1}	Diatoms	WA-PLS	0.75	0.49	Wachnicka *et al.* (2010)
Streams – U.S.A.*	0.010–3.100 mg L^{-1}	Periphyton	WA-PLS	0.80	0.50	Munn *et al.* (2002)
Streams – U.S.A.	0.016–0.560 mg L^{-1}	Diatoms	WA and WA-PLS		0.33–0.43	Ponader *et al.* (2008)
Peatland – U.S.A.	0.004–0.377 mg L^{-1}	Periphyton	WA-PLS	0.77	0.70	This Study
Peatland – U.S.A.	0.004–0.377 mg L^{-1}	Diatoms	WA-PLS	0.66	0.63	S. Hagerthey, unpubl. data
Nitrogen						
Lakes – Ireland	0.24–1.35 mg L^{-1}		WA	0.63–0.77		DeNicola *et al.* (2004)
Coastal Zone – U.S.A.	0.16–2.49 mg L^{-1}	Diatoms	WA-PLS	0.75	0.46	Wachnicka *et al.* (2010)
Coastal Zone – Finland	0.25–2.07 mg L^{-1}	Diatoms	WA-PLS	0.89	0.73	Weckström *et al.* (2004)
Streams – U.S.A.*	0.03–12.00 mg L^{-1}	Periphyton	WA-PLS	0.67	0.38	Munn *et al.* (2002)
Streams – Australia	0.04–23.60 mg L^{-1}	Diatoms	WA	>>0.60	>>0.35	Philibert *et al.* (2006)
Peatland – U.S.A.	0.41–8.15 mg L^{-1}	Periphyton	WA-PLS	0.60	0.46	This Study
Peatland – U.S.A.	0.41–8.15 mg L^{-1}	Diatoms	WA-PLS	0.36	0.31	S. Hagerthey, unpubl. data

Phosphorus and nitrogen were measured as total phosphorus and total Kjeldahl nitrogen, respectively, unless otherwise noted. Regression models are defined as WA for weighted averaging and WA-PLS for weighted averaging partial least squares.
*Phosphorus and nitrogen models were developed for orthophosphate and dissolved inorganic nitrogen, respectively.

producing a physiological response in algae (Tibby, 2004) or other factors limiting algal growth. For example, at high concentrations of P, nutrient limitation may shift to N or light (Grimshaw *et al.*, 1997). For our study, the lowest surface water N : P molar rations (<70) were associated with the underestimated residuals, indicating the potential shift from P to N limitation (data not shown).

Although the performance of the periphyton-TKN model developed for the Everglades ($r^2_{\text{jackknife}}$ = 0.46) was typical of published N models (Table 5), it was not as good as the salinity or TP models. Two factors limit the utility of an Everglades-specific N model for resource managers. First, the poor performance probably results from coincident N and P loading, which has been shown, in strongly P-limited systems, to adversely affect the predictive ability of N models (DeNicola *et al.*, 2004). Second, eutrophication in the Everglades results in a shift from a relatively open-water habitat to an emergent macrophyte-dominated wetland (Hagerthey *et al.*, 2008), which results in light, rather than nutrients, limiting periphyton production (Grimshaw *et al.*, 1997; Hagerthey, Cole & Kilbane, 2010).

Management application

Biological integrity is an integral component for evaluating the ecological integrity of aquatic ecosystems, especially in regulatory frameworks where biocriteria are being integrated with other criteria and standards programmes (Barbour *et al.*, 2000; Barbour & Paul, 2010). To attain biological criteria or objectives, water resource managers develop and implement measures to reduce or control the stressor(s) probably causing the biological impairment; thus, managers are most interested in knowing whether the management practice is having the desired biological effect independent of other factors. However, identity-based metrics, such as taxonomic composition and functional metrics (e.g. % sensitive taxa, % tolerant taxa), are not effective for directly assessing the effect of a management practice. This is due, in part, to the limited predictability caused by the lack of a mechanistic linkage with a particular stressor (Pollard & Yuan, 2010). Trait-based approaches are better suited for addressing specific management responses because traits are mechanistically connected to a stressor and are spatially universal and temporally stable (Pollard & Yuan, 2010; Culp *et al.*, 2011). The transfer functions developed for the Everglades are trait-based quantitative numerical methods and an ideal alternative because the abundances of species are modelled numerically in relation to a stressor (Birks, 2010). The resulting inferred value is a numerical representation of the stressor's effect on biological condition that can be compared against the management of the stressor independent of other factors. The benefits are that biological lags or hysteresis events can easily be identified and environmental conditions can be estimated when measurements are lacking.

The biological objective for the Everglades is the protection and maintenance of native flora and fauna (Everglades Forever Act, Florida Statute 373.4592 1994). In WCA-1, the diversion of polluted surface waters through constructed treatment wetlands (STA-1W and STA-1E) significantly reduced pollutant loading and also changed flow distributions (Table 3; Fig. 8). Relative to the operational period with minimal pollution control measures (Period A), periods with pollution control measures (Periods F &G) have reduced P loads by 86%, flow-weighted mean P concentrations by 36%, surface inflows by 64% and surface outflows by 48% (Table 3). These operational changes increased the proportion of precipitation inflows from 45 to 68%, shifting WCA-1 to rain-driven ecosystem. High salinity loading to the western perimeter of WCA-1 during periods C and D was associated with operational limitations resulting from the construction of infrastructure (Table 3 and Fig. 2). The transfer functions developed here provide managers with a tool to directly evaluate the biological response to efforts to reduce P and minimise high-salinity-canal-water intrusions.

Although practices have significantly reduced P, inferred P concentrations for sites close to the canal (X1,Z1, X2 and Z2) remained elevated (>0.018 mg L^{-1}) and did not decline with time, indicating that the periphyton did not reflect native assemblages (Fig. 8). Throughout the study period, marsh sites more than 2 km from the canal had inferred TP close to the benchmark, indicating that the periphyton assemblages were ecologically balanced. Wherein the inferred TP for interior marsh sites have not increased with time, current management efforts appear to be protecting the marsh from eutrophication. In addition, low inferred P concentrations following climatic induced short-term P pulses (e.g. hurricanes) do not appear to affect long-term biological properties (Fig. 8; Period E), indicating that periphyton assemblages in WCA-1 may be resilient to short-term P pulses.

Conductivity is typically used to assess the degree of canal-water intrusion into WCA-1 (Harwell *et al.*, 2008; Surratt *et al.*, 2008; Newman & Hagerthey, 2011). The inferred salinity patterns presented here indicate canal water penetrated into the marsh, causing a shift from ombrotrophic to minerotrophics assemblages at some sites (Fig. 7). Peak penetrations coincided with the construction of water and pollution control infrastructure that limited operations. However, upon completion, both measured and inferred salinity concentrations declined, indicating recovery of the ombrotrophic assemblage. For several of the sites, there was a noticeable lag between inferred and measured salinity concentrations during period F (Fig. 7) which may reflect a phase shift between salinity and periphyton response or a hysteresis caused by resilience and threshold effects.

It is assumed that conductivity dynamics are representative of other canal pollutants (Harwell *et al.*, 2008; Surratt *et al.*, 2008). Whilst our results support the use of surface water mineral concentrations as a tracer of canal water, extreme caution should be used to extend salinity patterns to other pollutants as causes of biological condition. For example, contrary to expectations, within site, salinity and TP were inversely related. This pattern was consistent among sites and for measured and inferred concentrations (Figs 7 and 8). The disconnect between TP and salinity in the marsh interior implies that local P dynamics are regulated by internal P loading (Carpenter, Ludwig & Brock, 1999; Sondergaard, Jensen & Jeppesen, 2003; Genkai-Kato & Carpenter, 2005) rather than canal-water intrusions. Therefore, it is important to monitor multiple stressors and biological attributes to provide managers with the information needed to establish the probable causal factors of ecological imbalance. Additional benefit would be gained through a better understanding of the mechanisms that lead to salinity-induced changes in ecosystem structure and function.

The management and operational decisions for WCA-1 are determined by a complex array of factors that include the hydrologic needs of the marsh, the need to implement flood control or water supply measures and the response to regional climatic events. Surratt *et al.* (2008) suggested that the negative ecological effects of pollutant loading from inflow structures could be minimised by discharging at low-to-moderate inflow rates, restricting the duration of high inflow events, balancing inflows with outflows and maintaining higher marsh stages relative to canals. Our results support these recommendations as evident by hydrologic conditions during 2006–09 (period G; Table 3). Reduced inflows and outflows increased the importance of precipitation, which achieved the ecological objective of minimising the negative effects in the south-western WCA-1 marsh associated with canal-water intrusions.

Periphyton-based transfer functions provided an effective biological assessment of water management actions to restore the Everglades. The combination of biologically inferred TP and salinity surface-water concentrations was readily used to identify valid causes of change and existence of degradation. The reporting of biological assessments in terms of well-defined water-quality metrics enables effective communication to resource managers. For the case of the Everglades, referencing biological inferred TP concentrations to the 0.010 mg L^{-1} numeric criterion provides managers with a metric to determine whether actions (e.g. project implantation or operation deviations) may be resulting in an imbalance or restoration of natural flora. More robust biological assessments can be obtained by combining indicator methods (e.g. transfer functions) with biological condition metrics (e.g. PIBI). Since both methods are based on measures of species abundance and traits of taxa, development of biological condition metrics should be relatively straightforward.

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References

Aloi J.E. (1990) A critical review of recent freshwater periphyton field methods. *Canadian Journal of Fisheries and Aquatic Science*, **47**, 656–670.

Barbiero R.P. (2000) A multi-lake comparison of epilithic diatom communities on natural and artificial substrates. *Hydrobiologia*, **438**, 157–170.

Barbour M.T. & Paul M.J. (2010) Adding value to water resource management through biological assessment of rivers. *Hydrobiologia*, **651**, 17–24.

Barbour M.T., Swietlik W.F., Jackson S.K., Courtemanch D.L., Davies S.P. & Yoder C.O. (2000) Measuring the attainment of biological integrity in the USA: a critical element of ecological integrity. *Hydrobiologia* **422/423**, 453–464.

Birks H.J.B. (1998) Numerical tools in palaeolimnology-progress, potentialities, and problems. *Journal of Paleolimnology*, **20**, 307–332.

Birks H.J.B. (2010) Numerical methods for the analysis of diatom assemblage data. In: *The Diatoms: Applications for the Environmental and Earth Sciences* (Eds J.P. Smol & E.F. Stoermer), pp. 23–54. Cambridge University Press, New York.

Birks H.J.B., Line J.M., Juggins S., Stevenson A.C. & ter Braak C.J.F. (1990) Diatoms and pH reconstructions. *Philosophical Transactions of the Royal Society of London Series B- Biological Sciences*, **327**, 263–278.

ter Braak C.J.F. (1986) Canonical correspondence analysis: a new eigenvector technique for multivariate direct gradient analysis. *Ecology*, **67**, 1167–1179.

ter Braak C.J.F. (1988) Partial canonical correspondence analysis. In: *Classification and Related Methods of Data Analysis* (Ed. H.H. Bock), pp. 551–558. North-Holland, Amsterdam.

ter Braak C.J.F. & Juggins S. (1993) Weighted averaging partial least squares regression (WA-PLS): an improved method for reconstructing environmental variables from species assemblages. *Hydrobiologia* **269/270**, 485–502.

Brandt L.A. (2006) *Benefits Anticipated from the 1995 Water Regulation Schedule for Water Conservation Area 1: Review and Analysis*. U.S. Fish and Wildlife Service, Boyton Beach.

Burkholder J.M. (1996) Interactions of benthic algae with their substrata. In: *Algal Ecology: Freshwater Benthic Ecosystems* (Eds R.J. Stevenson, M.L. Bothwell & R.L. Lowe), pp. 253–298. Academic Press Inc., San Diego, CA.

Carignan V. & Villard M.-A. (2002) Selecting indicator species to monitor ecological integrity: a review. *Environmental Monitoring and Assessment*, **78**, 45–61.

Carpenter S.R., Ludwig D. & Brock W.A. (1999) Management of eutrophication for lakes subject to potentially irreversible change. *Ecological Applications*, **9**, 751–771.

Culp J.M., Armanini D.G., Dunbar M.J., Orlofske J.M., Poff N.L., Pollard A. *et al.* (2011) Incorporating traits in aquatic biomonitoring to enhance causal diagnosis and prediction. *Integrated Environmental Assessment and Management*, **7**, 187–197.

DeNicola D.N., de Eyto E., Wemaere A. & Irvine K. (2004) Using epilithic algal communities to assess trophic status in Irish lakes. *Journal of Phycology*, **40**, 481–495.

Dixit S.S. & Smol J.P. (1994) Diatoms as indicators in the Environmental Monitoring and Assessment Program-Surface Waters (EMAP-SW). *Environmental Monitoring and Assessment*, **31**, 275–306.

Dolédec S. & Statzner B. (2010) Responses of freshwater biota to human disturbances: condition of J-NABS to development in ecological integrity assessments. *Journal of the North American Benthological Society*, **29**, 286–311.

Fritz S.C., Juggins S. & Battarbee R.W. (1993) Diatom assemblages and ionic characterization of lakes of the northern Great Plains, North America: a tool for reconstructing past salinity and climate fluctuations. *Canadian Journal of Fisheries and Aquatic Sciences*, **50**, 1844–1856.

Gaiser E. (2009) Periphyton as an indicator of restoration in the Florida Everglades. *Ecological Indicators*, **9S**, S37–S45.

Gaiser E., McCormick P.V., Hagerthey S.E. & Gottlieb A.D. (2011) Landscape patterns of periphyton in the Florida Everglades. *Critical Reviews in Environmental Science and Technology*, **41**, 92–120.

Gaiser E. & Rühland K. (2010) Diatoms as indicators of environmental change in wetlands and peatlands. In: *The Diatoms: Applications for the Environmental and Earth Sciences* (Eds J.P. Smol & E.F. Stoermer), pp. 473–496. Cambridge University Press, New York.

Gaiser E.E., Childers D.L., Jones R.D., Richards J., Scinto L.J. & Trexler J.C. (2006) Periphyton responses to eutrophication in the Florida Everglades: cross-system patterns of structural and compositional change. *Limnology and Oceanography*, **51**, 617–630.

Gasse F., Juggins S. & Khelifa L.B. (1995) Diatom-based transfer functions for inferring past hydrochemical characteristics of African lakes. *Palaeogeography Palaeoclimatology, Palaeoecology*, **117**, 31–54.

Genkai-Kato M. & Carpenter S.R. (2005) Eutrophication due to phosphorus recycling in relation to lake morphometry, temperature, and macrophytes. *Ecology*, **86**, 210–219.

Grimshaw H.J., Wetzel R.G., Brandenburg M., Segerblom K., Wenkert L.J., Marsh G.A. *et al.* (1997) Shading of periphyton communities by wetland emergent macrophytes: decoupling of algal photosynthesis from microbial nutrient retention. *Archiv für Hydrobiologie*, **139**, 17–27.

Guiot J. & de Vernal A. (2007) Transfer functions: methods for quantitative paleoceanography based on microfossils. In: *Development in Marine Geology*, *Volume* 1 (Eds C. Hillaire-Marcel & A. de Vernal), pp. 523–563. Elsevier, Amsterdam.

Hagerthey S.E., Bellinger B., Wheeler K., Gantar M. & Gaiser E. (2011) Everglades periphyton: a biogeochemical perspective. *Critical Reviews in Environmental Science and Technology*, **41**, 309–343.

Hagerthey S.E., Cole J.J. & Kilbane D. (2010) Aquatic metabolism in the Everglades: the dominance of net heterotrophy. *Limnology and Oceanography*, **55**, 653–666.

Hagerthey S.E. & Kerfoot W.C. (1998) Groundwater flow influences the biomass and nutrient ratios of epibenthic algae in a north temperate seepage lake. *Limnology and Oceanography*, **43**, 1227–1242.

Hagerthey S.E., Newman S., Rutchey K., Smith E.P. & Godin J. (2008) Multiple regime shifts in a subtropical wetland: establishing community specific thresholds to eutrophication. *Ecological Monographs*, **78**, 547–565.

Harwell M.C., Surratt D.D., Barone D.M. & Aumen N.G. (2008) Conductivity as a tracer of agricultural and urban runoff to delineate water quality impacts in the northern Everglades. *Environmental Monitoring and Assessment*, **147**, 445–467.

Hill B.H., Herlihy A.T., Kaufmann P.R., Stevenson R.J., McCormick F.H. & Johnson C.B. (2000) Use of periphyton assemblage data as an index of biotic integrity. *Journal of the North American Benthological Society*, **19**, 50–67.

King L., Barker P. & Jones R.I. (2000) Epilithic algal communities and their relationship to environmental variables in lakes of the English Lake District. *Freshwater Biology*, **45**, 425–442.

Lane C.M., Taffs K.H. & Corfield J.L. (2003) A comparison of diatom community structure on natural and artificial substrata. *Hydrobiologia*, **493**, 65–79.

Lane C.R. (2007) Assessment of isolated wetland condition in Florida using epiphytic diatoms at genus, species, and subspecies taxonomic resolution. *EcoHealth*, **4**, 219–230.

Marchant R., Norris R.H. & Milligan A. (2006) Evaluation and application of methods for biological assessment of streams: summary of papers. *Hydrobiologia*, **572**, 1–7.

McCormick P.V., Harvey J.W. & Crawford E.S. (2011) Influence of changing water sources and mineral chemistry on the Everglades ecosystem. *Critical Reviews in Environmental Science and Technology*, **41**, 28–63.

McCormick P.V. & Stevenson R.J. (1998) Periphyton as a tool for ecological assessment and management in the Florida Everglades. *Journal of Phycology*, **34**, 726–733.

McCune B. & Grace J.B. (2002) *Analysis of Ecological Communities*. MjM Software Design, Gleneden Beach, OR.

Munn M.D., Black R.W. & Gruber S.J. (2002) Response of benthic algae to environmental gradients in an agriculturally dominated landscape. *Journal of the North American Benthological Society*, **21**, 221–237.

Newman S. & Hagerthey S.E. (2011) Water Conservation Area 1- a case study of hydrology, nutrient, and mineral influences on biogeochemical processes. *Critical Reviews in Environmental Science and Technology*, **41**, 702–722.

Philibert A., Gell P., Newall P., Chessman B. & Bate N. (2006) Development of diatom-based tools for assessing stream water quality in south-eastern Australia: assessment of environmental transfer functions. *Hydrobiologia*, **572**, 103–114.

Pollard A.I. & Yuan L.L. (2010) Assessing the consistency of response metrics of the invertebrate benthos: a comparison of trait- and identity-based measures. *Freshwater Biology*, **55**, 1420–1429.

Ponader K.C., Charles D.F., Belton T.J. & Winter D.M. (2008) Total phosphorus inference models and indices for coastal plain streams based on benthic diatom assemblages from artificial substrates. *Hydrobiologia*, **610**, 139–152.

Potapova M. & Charles D.F. (2003) Distribution of benthic diatoms in U.S. rivers in relation to conductivity and ionic composition. *Freshwater Biology* **48**, 1311–1328.

Potapova M. & Charles D.F. (2007) Diatom metrics for measuring eutrophication in rivers of the United States. *Ecological Indicators*, **7**, 48–70.

Reed J.M. (1998) A diatom-conductivity transfer function for Spanish salt lakes. *Journal of Paleolimnology*, **19**, 399–416.

Reid M. (2005) Diatom-based models for reconstructing past water quality and productivity in New Zealand lakes. *Journal of Paleolimnology*, **33**, 13–38.

Ryves D.B., McGowan S. & Anderson N.J. (2002) Development and evaluation of a diatom-conductivity model from lakes in west Greenland. *Freshwater Biology*, **47**, 995–1014.

Sklar F.H., Chimney M.J., Newman S., McCormick P.V., Gawlik D.E., Miao S. *et al*. (2005) The ecological-societal underpinnings of Everglades restoration. *Frontiers in Ecology and the Environment*, **3**, 161–169.

Smol J.P. & Stoermer E.F. (Eds) (2010) *The Diatoms: Applications for the Environmental and Earth Sciences*. Cambridge University Press, New York.

Sondergaard M., Jensen J.P. & Jeppesen E. (2003) Role of sediment and internal loading of phosphorus in shallow lakes. *Hydrobiologia*, **506**, 135–145.

Stevenson R.J. (1996) An introduction to algal ecology in freshwater benthic habitats. In: *Algal Ecology: Freshwater Benthic Ecosystems* (Eds R.J. Stevenson, M.L. Bothwell & R.L. Lowe), pp. 3–30. Academic Press Inc., San Diego, CA.

Stevenson R.J. (2001) Using algae to assess wetlands with multivariate statistics, multimetric indices, and an ecological risk framework. in: *Biomonitoring and Management of North American Freshwater Wetlands* (Eds R.R. Rader, D.P. Batzger & S.A. Wissinger), pp. 113–140. John Wiley & Sons, San Francisco, CA.

Stevenson R.J., Pan Y., Manoylov K.M., Parker C.A., Larsen D.P. & Herlihy A.T. (2008) Development of diatom indicators of ecological conditions of streams of the western US. *Journal of the North American Benthological Society*, **27**, 1000–1016.

Stevenson R.J., Pan Y. & Van Dam H. (2010) Assessing environmental conditions in rivers and streams with diatoms. in: *The Diatoms: Applications for the Environmental and Earth Sciences* (Eds J.P. Smol & E.F. Stoermer), pp. 57–85. Cambridge University Press, New York.

Stumm W. & Morgan J.J. (1996) *Aquatic Chemistry: Chemical Equilibria and Rates in Natural Waters*. John Wiley & Sons Inc., New York.

Surratt D.D., Waldon M.G., Harwell M.C. & Aumen N.G. (2008) Time-series and spatial tracking of polluted canal water intrusion into wetlands of a national wildlife refuge in Florida, USA. *Wetlands*, **28**, 176–183.

Taukulis F.E. & John J. (2009) Development of a diatom-based transfer function for lakes and streams severely impacted by secondary salinity in the south-west region of western Australia. *Hydrobiologia*, **626**, 129–143.

Tibby J. (2004) Development of a diatom-based model for inferring total phosphorus in southeastern Australian water storages. *Journal of Paleolimnology*, **31**, 23–36.

Tibby J., Geil P.A., Fluin J. & Sluiter I.R.K. (2007) Diatom-salinity relationships in wetlands: assessing the influence of salinity variability on the development of inference models. *Hydrobiologia*, **591**, 207–218.

Tibby J. & Reid M.A. (2004) A model for inferring past conductivity in low salinity waters derived from Murray River (Australia) diatom plankton. *Marine and Freshwater Research*, **55**, 597–607.

Wachnicka A., Gaiser E., Collins L., Frankovich T. & Boyer J. (2010) Distribution of diatoms and development of diatom-based models for inferring salinity and nutrient concentrations in Florida Bay and adjacent coastal wetlands of south Florida (USA). *Estuaries and Coasts*, **33**, 1080–1098.

Weckström K., Juggins S. & Korhola A. (2004) Quantifying background nutrient concentrations in coastal waters: a case study from an urban embayment of the Baltic Sea. *Ambio*, **33**, 324–327.

Wetzel R.G. (2001) *Limnology: Lake and River Ecosystems*. Academic Press, New York.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Optima and Tolerances for the 319 taxa used to construct transfer functions.

Table S2. Measured and inferred (arithmetic mean ± SD) salinity and TP concentrations for operational and climatic periods for sites in L-7 canal and WCA-1 marsh.

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