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The effects of acid sulphate run-off on a subtidal estuarine macrobenthic community in the Richmond River, NSW, Australia

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The Richmond River is periodically exposed to acid sulphate run-off (ASR). Leaching of acidified water into this system commonly arises from infiltration of rainwater through acid sulphate soils that have been oxidized during construction of flood mitigation drains. Leaching events have been linked to major fish kills and can also lead to sublethal effects in fish, such as red spot disease. The effects of ASR on subtidal estuarine macrobenthic assemblages in the region of the Richmond known as the Tuckean Broadwater are examined here. Canonical correspondence analysis revealed that water transparency, pH, and soluble aluminium concentration were the most significant contributors to variation in macrobenthic community structure. Partial canonical correspondence analysis was performed to examine the effects of factors associated with ASR on the relative abundance of macrobenthic species, in isolation from the influence of other environmental variables. Variation in relative abundance was not significantly related to the individual effects of either soluble aluminium or pH. However, their combined effect on species abundance was significant, reflecting that macrobenthos at this site responded to chemical speciation of aluminium at certain pH ranges, rather than directly to either pH or soluble aluminium concentration. Results also showed that two species of sub-surface deposit-feeding polychaetes, *Nephtys australiensis* and *Notomastus torquatus*, were the most sensitive to the chemical speciation of aluminium and have tolerances for aluminium species that occur at different levels of acidification. The feeding activities of these two species may have removed the iron floc formed on the sediment surface under acidified conditions, thereby providing suitable conditions for other macrobenthic species to recolonize the Tuckean Broadwater.

Key words: acid sulphate run-off, canonical correspondence analysis, chemical speciation, direct gradient analysis, estuary, indicator species, macrobenthos, partial-constrained ordination, pH, soluble aluminium concentration.

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Introduction

Anthropogenic acidification of streams, lakes, and upper estuaries is a worldwide phenomenon that has only recently received close attention. Although the source of acidification in these systems varies from place to place, the consequences are of increasing concern to ecologists, environmental managers, and other interest groups. In the United States and Canada, acidification of waterways is primarily due to acid rain (Gorham *et al*., 1984; Herczeg *et al*., 1985; Kelly *et al*., 1984; Lonergan and Rasmussen, 1996; Malley and Chang, 1985; Nero and Schindler, 1983; Schindler *et al*., 1985). In Australia and parts of Southeast Asia, however, acid sulphate run-off (ASR) is the major contributor to the acidification of waterways (Roach, 1997). This mode of acidification begins with oxidation of iron pyrite within coastal lowland soils. This produces sulphuric acid, which can leach into waterways, particularly after heavy rain. Exposure of pyrite to atmospheric oxygen can occur naturally through tectonic uplift (Roach 1997) or through lowering of the water table during dry periods (Ferguson and Eyre, 1995). However, ASR has been exacerbated owing to the construction of flood mitigation drains and aquaculture ponds in areas where potential acid sulphate soils were present (Alongi, 1998; Roach, 1997). Where such construction has taken place, severe deterioration of water quality, particularly with regard to pH and soluble aluminium concentrations, has led to appreciable declines in the fitness of fauna

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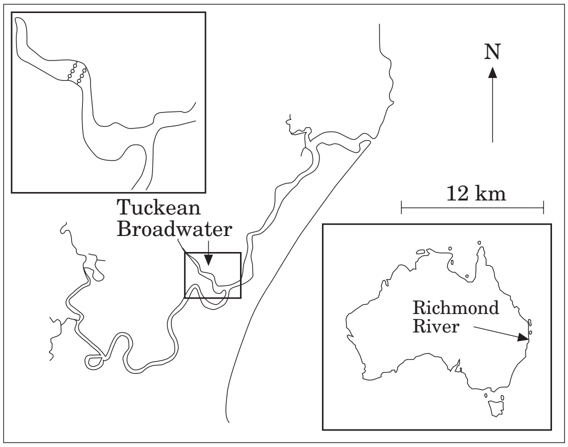

Figure 1. Location map of the Tuckean Broadwater study site in relation to the entire Richmond River and the Australian continent. Inset is a schematic representation of how stations were arranged within the parallel cross-channel transects.

inhabiting adjacent waterways and the ponds themselves (Ferguson and Eyre, 1995; Neal, 1993; Roach, 1997; Sammut *et al*., 1996). In some instances, this has created conflict between fisheries and agricultural interest groups (Sammut *et al*., 1996).

Acidificiation has both lethal and sublethal effects. For example, ASR can lead to gill damage and subsequently to mass fish kills, but it also increases the susceptibility of surviving fish to red spot disease (Roach, 1997). ASR has also been linked to lowered aquaculture productivity of prawns in some Southeast Asian countries (Alongi, 1998). This may be because acidification-related factors inhibit calcium uptake during the moulting process, which may in turn increase moulting time or cause exoskeletons to become softer in post-moult prawns (Malley and Chang, 1985).

The Richmond River is an estuarine system in which many fish kills associated with ASR have been reported (Neal, 1993), and a considerable amount of work has been done on the nature of the ASR phenomenon (Ferguson and Eyre, 1995; Lin *et al*., 1996; Neal, 1993; Roach, 1997; Sammut *et al*., 1996). The Richmond is a bar-built estuary situated in northern New South Wales, Australia (28°52′S, 153°34′E), with a catchment area of 6940 km^2 (Fig. 1); 15% of the catchment area is coastal flood plain, which supports a range of uses, particularly sugar-cane growing and cattle farming. The river itself supports productive commercial and recreational fisheries, which both target a range of species (Roach, 1997). There is a perception among these fisheries groups that ASR has contributed to declining catch rates (Sammut *et al*., 1996).

Although several ASR hot spots have been identified in the Richmond River (Ferguson and Eyre, 1995; Lin *et al*., 1996; Roach, 1997; Sammut *et al*., 1996), most of the sites listed are either subject to short-term and infrequent disturbance from ASR or are poorly represented in terms of their macrobenthos. The Tuckean Broadwater is the only region of the Richmond in which a broad range of macrobenthic species are exposed to sustained periods of ASR. Accordingly, this study focused entirely on the impact of ASR on macrobenthos in this region.

The aims were to determine: (a) which of the mesured environmental factors are important in structuring macrobenthic assmblages of the Tuckean Broadwater; (b) which, if any, of the factors associated with acidification significantly affect species abundance; (c) the nature of species responses to pH and soluble aluminium; and (d) which species, or combination of macrobenthic species, are the best local indicators of acidification.

Methods

The Tuckean Broadwater is situated in an area originally known as the Tuckean Swamp, which covered approximately 120 km^2 prior to the implementation of flood plain management measures. These measures included draining the swamp, removing a large area of mangroves, construction of a barrage to prevent saltwater intrusion, and the establishment of agriculture and flood mitigation drains. The river section below the barrage is shallow (generally between 0.5 m and 3 m depth), is subject to the intrusion of estuarine water, and is bordered by some of the largest stands of *Avicennia marina* and *Brugiera gymnorhiza* mangroves along the river. It is a feeding and roosting site for birds such as pelicans, sea eagles, brahmany, and whistling kites and is thought to be a major nursery area for economically important fish species (Ferguson and Eyre, 1995; Neal, 1993; Roach, 1997).

Benthic sampling was conducted every three months between January 1996 and October 1997 in concurrence with an overall study of macrobenthic community dynamics along the entire estuarine gradient. This sampling timetable was chosen to coincide with seasonal changes and for logistical reasons. Three replicate samples were collected at three stations (east bank, middle, and west bank) along two cross-channel transects, approximately fifty metres apart (Fig. 1). This regime was chosen to account for significant cross-channel variation in terms of substratum type, depth, and hydrology, as well as mesoscale variation within the study site.

Samples were collected using a 0.05 m^2 Van Veen grab, and all organisms retained in 1 mm mesh sieves were removed with fine forceps, placed in calico bags with a waterproof label, and submersed in a drum containing a 5% (v/v) formalin solution in sea water. Bags were later transferred into a drum containing 70%

(v/v) ethanol in the laboratory and were subsequently identified to species level where possible, using a stereo-dissector microscope, relevant keys (Hutchings and Murray, 1984; Moverley, 1986; Robinson and Gibbs, 1982) and advice from taxonomic experts. The abundance of each species was recorded for each individual sample.

Prior to sieving, five sediment redox potential measurements were recorded from sediment containing benthos, using a redox probe described in Kerr and Corfield (1998). A sediment sample was also collected at each station, using the Van Veen grab. The silt-clay fraction (<63 μm) was determined by wet sieving and the particle size distribution of the coarser material using dry sieving methods (Buchanan and Kain, 1971). Organic carbon content was determined using a LECO CR-12 carbon analyser. A 50:50 mixture of sediment from the two smallest retained sediment fractions was used for carbon analysis, as much of the organic carbon in these sediment grades is labile and as such, was potentially available for consumption by deposit-feeding macrobenthic organisms at the time of sampling (Gaston, 1987). This method also eliminated the need for acidifying the samples prior to analysis, because negligible amounts of $CaCO_3$ (in the form of shell grit) occur within these fractions.

Dissolved oxygen content, pH, conductivity, and water temperatures were measured at each station using a Horiba U-10 multiprobe that was calibrated before and after sampling. Turbidity was measured using a standard Secchi desk, while bottom depth was estimated from the graduations on the Secchi disk rope. Two water samples were collected on each occasion to determine soluble aluminium concentrations. These were placed in 300 ml acid-washed polypropylene containers to prevent contamination of samples from extraneous sources of Al^{3+}. All water quality measurements and water samples were taken at a standardized depth range between 0.5 m and 1.0 m. Prior to laboratory analysis, water samples were stored in refrigerated conditions, filtered using 45 μm Millipore filters, and acidified with 1 ml of 5M nitric acid. They were then analysed using flow injection analysis on a LACHAT xyz sampler device.

Direct gradient analyses were used to evaluate the relative importance of various environmental factors in determining species composition and the nature of species–environment relationships. Prior to performing these analyses, replicate benthic samples were pooled within stations in order to equate the number of samples in the biotic data with the number of environmental data samples. Environmental data were also standardized prior to analysis such that each data point represented a mean value of a particular environmental parameter for an individual station. Granulometric measures were arcsin transformed to ensure that data for these parameters were normally distributed.

Several forms of direct gradient analysis were applied. First, redundancy analysis (RDA) and canonical corre-spndence analysis (CCA) were carried out on complete environmental and biotic data sets to establish which provided the optimum model to describe the response of species to measured environmental variables. Prior to each of these analyses, a manual forward selection procedure was used to select factors for inclusion as terms in these models. This ensured that the proportion of variation in the biotic data explained by CCA and RDA models was not simply due to the additive effect of increasing the number of explanatory variables. In both cases, species data were $\log_{10}$ (x +1) transformed and the abundance of rarer species was downweighted, to avoid the confounding influence of such species.

Secondly, partial CCA was used to determine the potential suitability of macrobenthos as indicators of ASR. In this analysis, both the individual and combined influence of pH and soluble aluminium concentration on the relative abundance of certain macrobenthic species was assessed in isolation from the effects of other measured environmental factors. This was achieved by including the other measured factors as covariables in these analyses. Although ASR normally occurs in conjunction with freshwater discharge, flow variability measurements were unavailable and therefore could not be included as covariables.

The macrobenthos included in the partial CCA model were relatively common species for which more than 5% of the variation in abundance was explained directly by either pH or soluble Al^{3+} concentration (i.e., r^2 of linear regressions between abundance and either factor >0.05). Rare species (those occurring in <5% of all samples) were omitted because they are poorly suited as potential biological indicators (Lonergan and Rasmussen, 1996). Under this criterion, only four species were selected and these were similar in terms of numerical abundance. Thus no transformation or downweighting of species data was performed on biotic data prior to partial CCA. Systematic removal of species from the biotic data set was carried out in order to obtain the optimum fit for the model and thereby determine the species most sensitive to ASR-related factors.

Restricted Monte Carlo permutation tests were performed as part of the manual forward selection process and as a way of determining the significance of Eigen-values derived from the final RDA, CCA, and partial CCA models. Restricted permutations favoured the null model (completely random permutations) because benthic samples were collected in a time-series fashion. Under this permutation scheme, only samples collected during the same sampling occasion were permuted. All direct gradient analyses were carried out using CANOCO for Windows version 4.0, and species-environment relationships were displayed graphically as point-vector biplots created in the complementary

Table 1. Results of RDA and CCA.

Axis	RDA 1	RDA 2	RDA 3	RDA 4	CCA 1	CCA 2	CCA 3	CCA 4
Eigenvalues	0.108	0.073	0.049	0.025	0.442	0.135	0.110	0.478
Sp.-env. corr.	0.77	0.77	0.69	0.60	0.88	0.73	0.69	0.00
% sp. data	10.8	18.1	23.0	25.5	20.2	26.4	31.5	53.3
% sp.-env.	42.4	70.9	90.0	100.0	64.3	84.0	100.0	0.00
Test on 1st axis								
Eigenvalue	0.108				0.442			
F-ratio	4.124				8.870			
p-value	0.090				0.020			
Test on all axes								
Trace	0.255				0.688			
F-value	2.910				5.353			
p-value	0.350				0.015			
Factors included	pH SECH ALUM PERD				pH SECH ALUM			

% sp. data, cumulative % variance in species data explained; % sp.-env., cumulative % of species–environment relationship with increased number of dimensions; trace, sum of all canonical eigenvalues. Tests on axes are based on restricted Monte Carlo permutations, while factors included in each model were chosen using manual forward selection. SECH, turbidity; ALUM, soluble Al^{3+} concentration; PERD, very fine sand (125 μm).

graphics package CanoDraw 3.1 (ter Braak and Smilauer, 1998).

Results

Larger Eigenvalues were derived from CCA than from RDA (Table 1), which indicates that macrobenthic species in the Tuckean Broadwater display a predominantly unimodal response to major environmental gradients. In the light of this finding, further discussion of results pertaining to direct gradient analysis will refer only to those derived from CCA or partial CCA. Of the 15 environmental variables measured, only three (pH, soluble aluminium concentration, and turbidity) were chosen by forward selection for inclusion in the final CCA model.

Results of Monte Carlo permutations tests on the first CCA ordination axis confirm the existence of a dominant environmental gradient, while tests on the trace (sum of all canonical Eigenvalues) indicate that the influence of pH, soluble aluminium concentration, and turbidity on macrobenthic community structure was significant (Table 1). Some 100% of the variability within these environmental parameters is described by the first three CCA ordination axes, and nearly one third (31.5%) of the variation in the biotic data is described by the combined influence of pH, soluble aluminium concentration, and Secchi depth (Table 1). The point-vector biplot of these results indicates that pH and soluble Al^{3+} concentration are highly correlated and that these two factors form the dominant gradient along the first (x) axis (Fig. 2). This biplot also indicates that the turbidity gradient is uncorrelated with the ASR-related gradient,

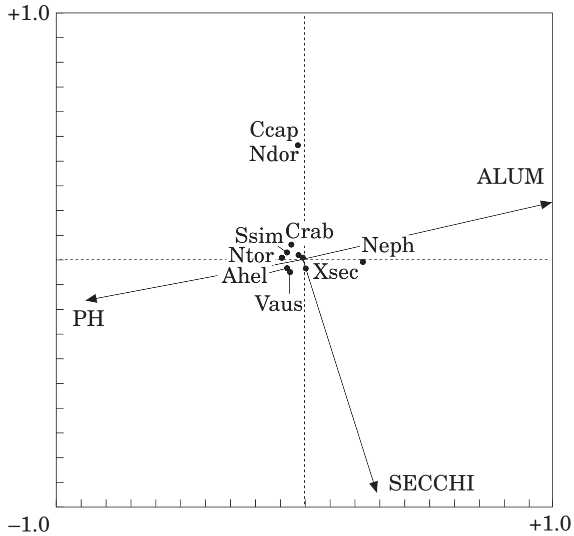

Figure 2. Point-vector plot of the relationship between weighted species abundances and the measured environmental parameters selected by manual forward selection for inclusion in the CCA model (pH; Secchi, turbidity; Alum, soluble Al^{+3} concentration). For the sake of clarity, some species scores are not labelled. Points in plots: Ahel=*Arthritica helmsii*; Crab, *Halicarcinus* sp.; Ndors, *Nassarius dorsatus*; Nepht, *Nephtys australiensis*; Ntorq, *Notomastus torquatus*; Vaust, *Victoriopisa australiensis*; Ssim, *Scoloplos scoloplos simplex*; Xsec, *Xenostrobus securis*; Ccap, *Capitella capitata*.

given the angle between vectors. Few of the species scores are far from the origin, suggesting that most of the thirteen species found were either weakly affected by these three environmental factors or were more

Table 2. Results of partial-CCA for the final models, in which only two species (*N. torquatus* and *N. australiensis*) were included.

ASR factor test on	pH 1st axis	Al^{3+} 1st axis	pH and Al^{3+} combined 1st axis	pH and Al^{3+} combined All axes
Trace	0.001	0.000	n.a.	0.089
F-ratio	0.471	0.007	30.409	15.205
p-value	0.440	0.910	0.005	0.005
% variation explained	2.4	0.0	61.5	

Trace, the sum of all canonical eigenvalues. Restricted Monte Carlo permutations tests were applied to the 1st axis only, when but one environmental variable was used and to all axes when both pH and Al^{3+} were included in the model.

abundant where pH, soluble aluminium concentration, and water transparency levels were not extreme.

Of the species collected, only four were considered as potential indicator species, based on their relative abundance and the strength of the relationship between abundance and pH or soluble Al^{3+} concentration. These were the capitellid *Notomastus torquatus*, the nephtyid *Nephtys australiensis*, the bivalve *Arthritica helmsii* and a tube-dwelling, melitid amphipod, *Victoriopisa australiensis*. If the effects of all other measured environmental factors were accounted for, the individual effects of pH and soluble Al^{3+} concentration on relative species abundance were not significant. This was not true if both factors were included as environmental variables in the partial CCA model (Table 2). Results obtained *via* systematic removal of species from the model indicate that *N. torquatus* and *N. australiensis* were the species most sensitive to the combined influence of pH and soluble aluminium concentration. In this model, pH and soluble aluminium concentration account for nearly two thirds (61.5%) of the variation in the abundance of these polychaetes (Table 2). The relationship between abundance of *N. torquatus* and *N. australiensis* and the pH–soluble aluminium concentration gradient is displayed in Figure 3. The relative position of the two species scores in relation to vectors representing pH and soluble aluminium concentration reflects a different response to forms of bio-available Al^{3+} that occur at different levels of acidification.

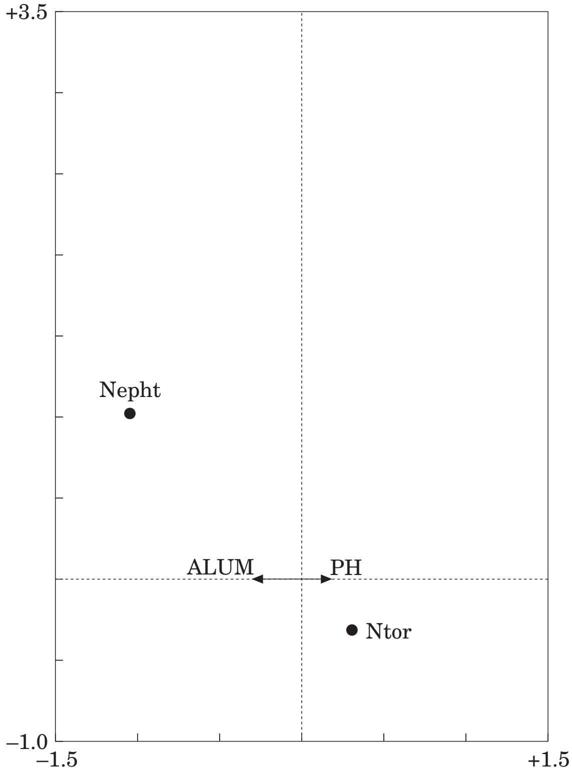

Figure 3. Point-vector plot of the relationship between the weighted abundances of the polychaetes *N. torquatus* and *N. australiensis* and the two ASR-related variables (pH and soluble Al^{3+}) once covariables had been accounted for in the partial CCA model.

Discussion

The occurrence of ASR as either a pulse or a chronic disturbance event has made study and management of ASR impacts on estuarine fauna difficult. Roach (1997) suggested that the impact of a disturbance can only be considered significant when its direct effects on community structure are greater than those of other biotic and abiotic factors. Nevertheless, he was unable to decouple the effects of flooding and ASR using his approach to monitoring the effects on macrobenthic assemblages. Our results show that ASR-related factors form the dominant environmental gradient affecting the relative abundance of macrobenthic species and were among three factors that were globally significant in determining macrobenthic species composition in the Tuckean Broadwater. Furthermore, suitable indicator species (i.e., those affected predominantly by ASR) and the mechanism by which these organisms are affected were also determined using direct gradient analysis techniques.

Turbidity was also among the most significant contributors to variation in the relative abundance of macrobenthos in the Tuckean Broadwater. Although turbidity decreases substantially during ASR (Ferguson and Eyre, 1995; Roach, 1997; Sammut *et al*., 1996), the turbidity gradient was not correlated with the ASR gradient in terms of its influence on macrobenthic community dynamics. This suggests that macrobenthos at this site responded to changes in turbidity generated by wind-driven resuspension of fine sediment particles, rather than the flocculation of suspended particles during ASR. A marked increase in the relative abundance of the polychaete *Capitella capitata* and the dog whelk *Nassurius dorsatus* in response to low water transparency is evident in Figure 2. However, this result probably reflects a positive response of these species to increases in the quantity of decaying plant material as a result of decreases in the amount of light available for photosynthesis, rather than a direct response to decreased water transparency. McKillup and McKillup (1997) also noted large increases in the abundance of *N. dorsatus* in response to increased food availability and suggested that this dogwhelk may even affect macrobenthic species composition, through feeding-related disturbance on the sediment surface.

Favourable results produced by CCA compared with RDA in terms of explaining variability within the biotic data suggests that macrobenthic species in the Tuckean Broadwater exhibit a predominantly unimodal response to the major environmental gradients. This indicates that they have distinct habitat preferences for, or narrow tolerance limits to, factors associated with important environmental gradients. RDA is only appropriate where gradients are short and tolerance limits are approximately as broad as the measured gradient length (ter Braak and Smilauer, 1998), or in other words, where dose-response reactions to environmental gradients occur. This situation is said to occur rarely (Borcard *et al*., 1992; ter Braak, 1986), but has been observed in studies by Lonergan and Rasmussen (1996) and Legendre and Anderson (1999).

Partial CCA revealed that pH and soluble Al^{3+} concentration significantly affect the relative abundance of macrobenthos in synergy, though not individually. This implies that macrobenthos in the Tuckean Broadwater respnds to chemical speciation of aluminium with varying pH, rather than in a dose-response fashion, to variation in pH and soluble Al^{3+} concentration. This finding is in agreement with that of Malley and Chang (1985), who found that monomeric aluminium occurring at a pH of 5.5 was responsible for reduced calcium uptake in post-moult crayfish. The optimum species-environment model described by partial CCA indicated that *N. australiensis* and *N. torquatus* were the species most sensitive to chemical speciation of aluminium with varying pH. Thus, these species are the most effective indicators of ASR in the Tuckean Broadwater. Figure 3 shows that *N. australiensis* has a tolerance of, or preference for, a form of aluminium that occurs at lower pH compared with that favoured by *N. torquatus*.

Chemical speciation of aluminium with varying pH was not examined here, but results of a study by Ferguson and Eyre (1995) suggest that *N. australiensis* is probably tolerant of monomeric aluminium (Al^{3+}), which only occurs under severe acidification, while *N. torquatus* is tolerant of aluminium hydroxides and aluminium sulphates, which occur when ASR is either moderate or associated with heavy freshwater discharge. Thus, the occurrence of soluble aluminium as either of these forms explained 61.5% of variation in the relative abundance of the two polychaetes. Because the acidity in the Tuckean Broadwater is determined by whether ASR occurs as a persistent or an episodic event, increases in the relative abundance of either of these polychaetes may, in the absence of ongoing water quality measurements, provide a good indication of the degree of exposure to ASR at this location.

Indirect effects of ASR on the assemblage were not examined, because this would have required a manipulative field experiment, rather than a monitoring approach. An example of a potential indirect effect of ASR on macrobenthos was put forward by Roach (1997), who noted an iron precipitate on the sediment surface in the Tuckean Broadwater after ASR and believed that this might postpone recovery by impeding larval settlement. He also suggested that recolonization of this site might only be successful upon the removal of this precipitate and that this may require either high-flow conditions sufficient for sediment scouring, or dredging of the benthic habitat. Observations in the Tuckean Broadwater by Sammut *et al*. (1995) confirm the presence of iron floc on the sediment surface in the form of a hard crust, particularly at times coinciding with persistent ASR. However, there is no indication that low diversity and abundance of macrobenthos persisted after major ASR episodes, despite the absence of high flow conditions required for sediment scouring (Corfield, 1999). This indicates that recovery was not severely inhibited by the iron floc. Given that the both ASR-tolerant polychaetes are deposit feeders, their sediment reworking may have been responsible for mediating the recovery process.

While direct gradient analyses were used here to establish ecologically important species–environment relationships, the causal nature of such relationships can only be determined in manipulative experiments or exotoxicological studies, because this multivariate technique assumes that environmental factors affect species composition in a non-mutualistic manner (Borcard *et al*., 1992). Direct gradient analysis was used here in an exploratory capacity, and hypotheses generated from these results provide a narrower focus for determining the causative effects of ASR-related factors on the status of macrobenthic assemblages in the Tuckean Broadwater and elsewhere. Although the results presented here are based on only two years of data obtained from a single impacted site, there is great potential for the use of *N. australiensis* and *N. torquatus* as indicators of ASR at other locations, because these two species occur widely within Australian estuaries.

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