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A New Approach for Detecting and Mapping Sewage Impacts

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Increased nitrogen loading has been implicated in eutrophication occurrences worldwide. Much of this loading is attributable to the growing human population along the world's coastlines. A significant component of this nitrogen input is from sewage effluent, and delineation of the distribution and biological impact of sewage-derived nitrogen is becoming increasingly important. Here, we show a technique that identifies the source, extent and fate of biologically available sewage nitrogen in coastal marine ecosystems. This method is based on the uptake of sewage nitrogen by marine plants and subsequent analysis of the sewage signature (elevated $\delta^{15}N$) in plant tissues. Spatial analysis is used to create maps of $\delta^{15}N$ and establish coefficient of variation estimates of the mapped values. We show elevated $\delta^{15}N$ levels in marine plants near sewage outfalls in Moreton Bay, Australia, a semi-enclosed bay receiving multiple sewage inputs. These maps of sewage nitrogen distribution are being used to direct nutrient reduction strategies in the region and will assist in monitoring the effectiveness of environmental protection measures. © 2001 Elsevier Science Ltd. All rights reserved.

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The nitrogen cycle is one of Earth's most important elemental cycles, and also the most influenced by human activity (Heaton, 1986). A significant component of marine eutrophication in many near shore environments can be attributed to anthropogenic inputs of sewage nitrogen (Lee and Olsen, 1985; Nixon *et al.*, 1986). Managing and monitoring the effects of sewage entering marine ecosystems has become a major environmental challenge (Nixon, 1995). The distribution of sewage effluent in marine ecosystems can be mapped with such parameters as salinity, dissolved nutrients, bacteria, organic matter composition, radioisotope tracers, dye fluorescence, water current measurements and $\delta^{15}N$ ratios in water and sediment (Smith-Evans and Dawes, 1996; Lindau *et al.*, 1989; Sweeney *et al.*, 1980). These techniques are useful for determining the physical extent of sewage effluent, but provide little insight into the biological uptake and impact of sewage nutrients. Furthermore, temporal and spatial variability confounds interpretation of these parameters. We have developed a technique, involving nitrogen stable isotopes in marine plants, that provides temporally integrated information on the biologically available and therefore ecologically significant, component of sewage nitrogen.

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Nitrogen Stable Isotopes

There are two naturally occurring atomic forms of nitrogen. The common form that contains seven protons and seven neutrons is referred to as nitrogen 14 and is expressed as ^{14}N. A heavier form that contains an extra neutron is called nitrogen 15 and is expressed as ^{15}N. By measuring the ratio of ^{15}N to ^{14}N in dried plant tissue and comparing to a worldwide standard, the relative amount of ^{15}N, or $\delta^{15}N$ in the plant can be determined, as described below:

$$\delta^{15}N(‰) = \left(R_{sample}/R_{standard} - 1\right) \times 10^3,$$

where R is defined as the atomic $^{15}N/^{14}N$ ratio. The global standard used is atmospheric N_2. The isotopic composition of atmospheric N_2 is considered to be uniform with a ^{15}N abundance of 0.3663% (Junk and Svec, 1958; Sweeney *et al.*, 1978; Mariotti, 1983).

The various sources of nitrogen pollution to coastal ecosystems often have distinguishable $^{15}N/^{14}N$ ratios, thereby providing a means to identify the source of pollution (Heaton, 1986). For example, nitrogen fertilizer and sewage-derived nitrogen have distinct differences in their $\delta^{15}N$ signatures. The main method of nitrate and ammonium fertilizer production is by industrial fixation of atmospheric nitrogen, resulting in products that have $\delta^{15}N$ values close to zero. However in animal or sewage waste, nitrogen is excreted mainly in the form of urea, which when hydrolyzed, produces a temporary rise in pH. The more basic conditions favour conversion to ammonia, which is easily lost by volatilization to the atmosphere. Fractionation during this

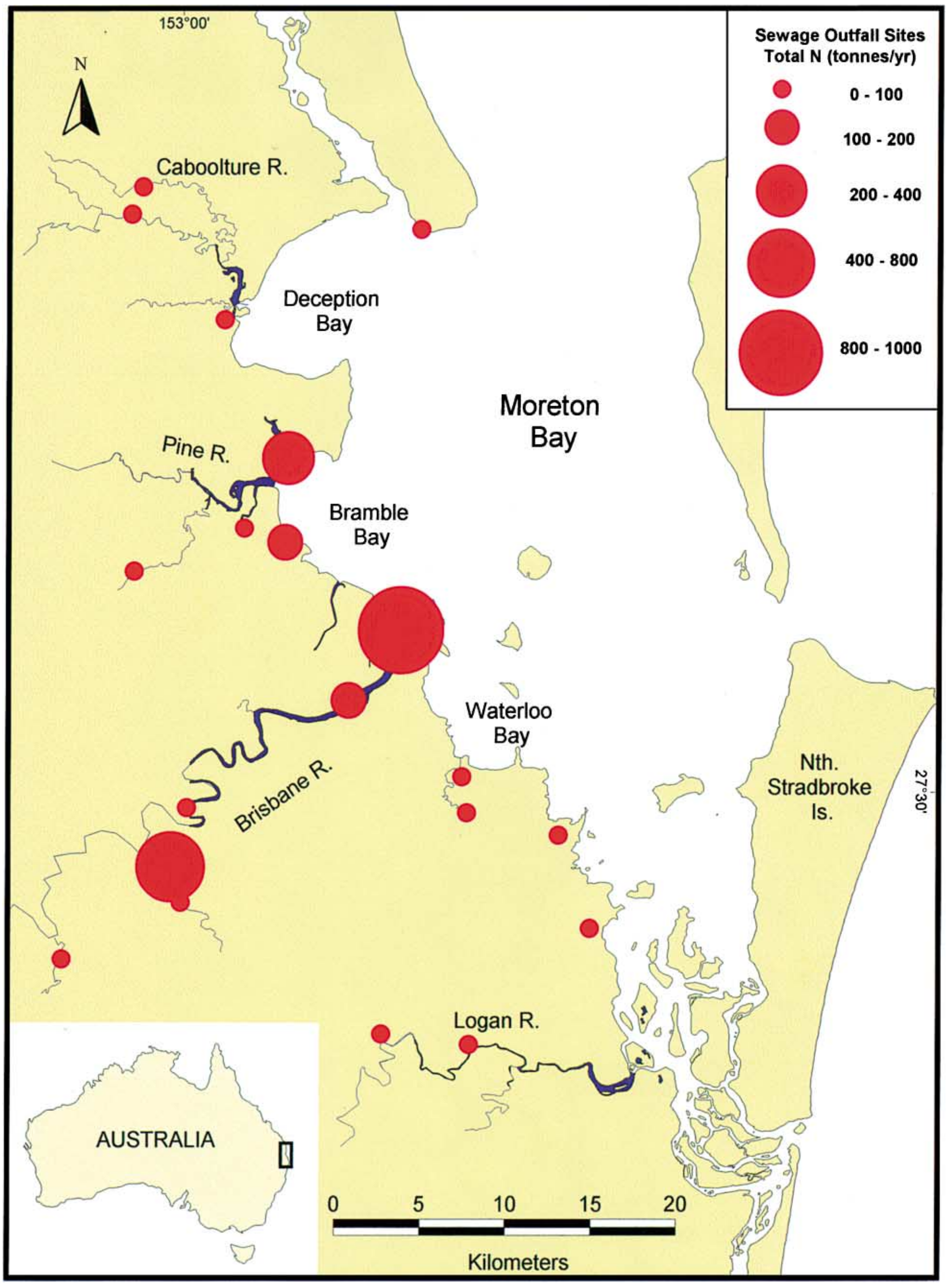


Fig. 1 Location and nitrogen loading (tonnes/yr) of sewage effluent outfalls into Moreton Bay and tidal estuaries.

process results in the ammonia, which is lost from the system, being depleted in ^{15}N. The remaining ammonium, now correspondingly enriched in ^{15}N, is subsequently converted to ^{15}N-enriched nitrate, which is more readily leached and dispersed by water (Heaton, 1986). The elevated $\delta^{15}N$ signature of treated sewage (~10‰) therefore distinguishes it from other nitrogen sources entering marine ecosystems (cf. fertilizer nitrogen ~0‰) (Heaton, 1986).

Sewage Nitrogen and Marine Plants

Marine plants can absorb and assimilate sewage-derived nitrogen, their tissue $\delta^{15}N$ reflecting exposure to sewage over a given timeframe (Peterson and Fry, 1987). Elevated $\delta^{15}N$ signatures have been identified in marine plants exposed to seabird guano (Wainright *et al.*, 1998), septic contaminated groundwater (McClelland *et al.*, 1997) and sewage effluent (Cabana and Rasmussen, 1996; Hansson *et al.*, 1997; Hobbie and Fry, 1990; Grice *et al.*, 1996; Udy and Dennison, 1997). Variations of $\delta^{15}N$ in naturally occurring marine plants adjacent to sewage outfalls, therefore, provides a technique for detecting and mapping the geographical extent of biologically available sewage nitrogen.

In many cases, however, submersed marine vegetation has disappeared in regions impacted by sewage.

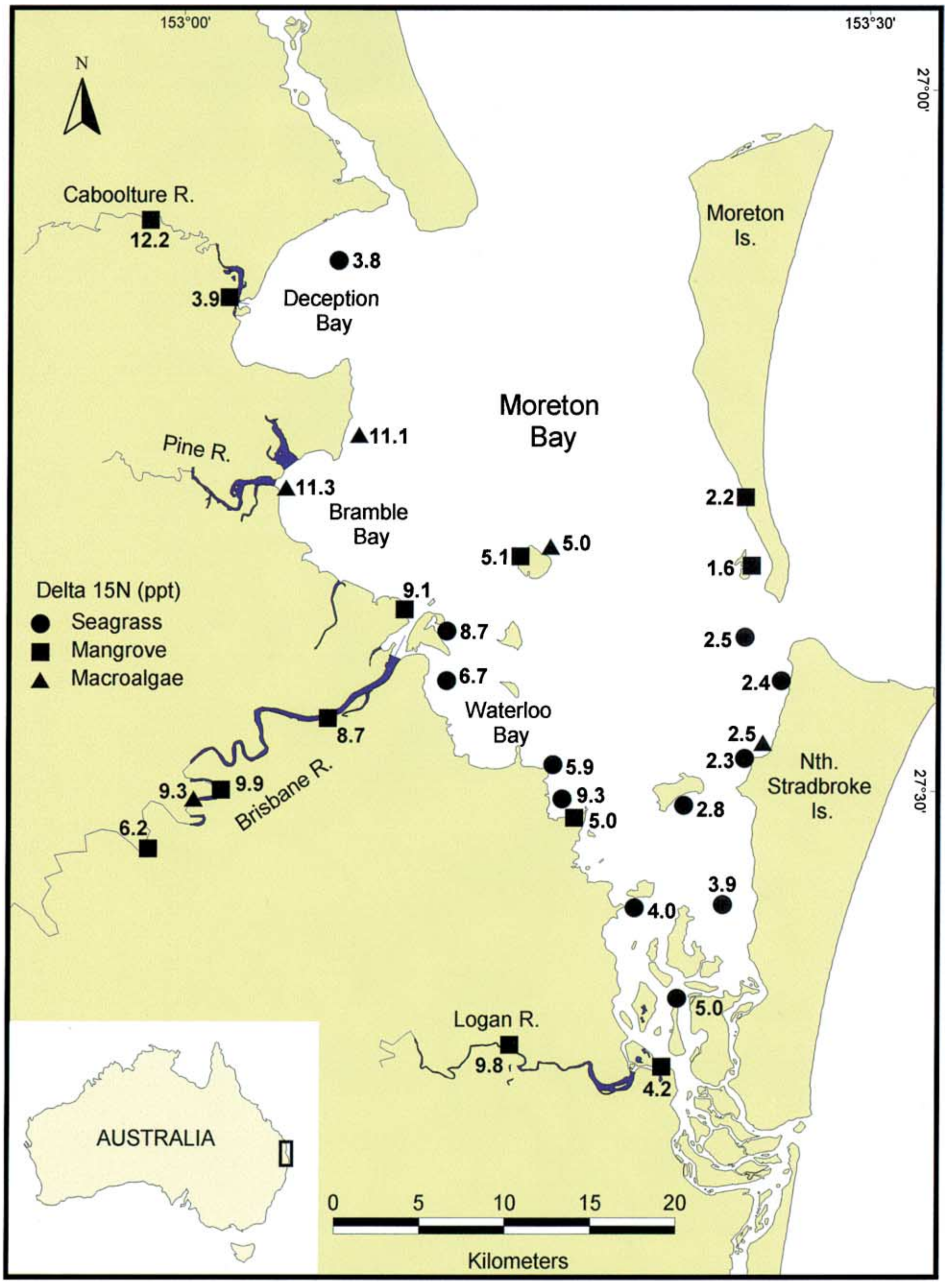


Fig. 2 δ^{15}N values of naturally occurring marine plants (mangroves, seagrass and macroalgae) throughout Moreton Bay and tidal estuaries.

To map the fate of sewage derived N within these regions, a method was developed which utilized changes in the δ^{15}N values of macroalgae deployed *in situ* at any desired location. This technique of mapping sewage nitrogen with naturally occurring and deployed marine plants was tested in Moreton Bay, QLD, Australia.

Study Region

Moreton Bay, on the east-coast of Australia (27°15′S, 153°15′E), is a sub-tropical, shallow coastal embayment (1.5×10^3 km^2). Its drainage catchment (2.1×10^4 km^2) contains an urban center with a population of approximately 1.5 million people. Development is concentrated on the western side of the bay and high-nutrient concentrations in surrounding waters reflect this urban influence (Dennison and Abal, 1999). The majority of treated sewage effluent is discharged into four river estuaries on the western side of Moreton Bay, with over 50% discharged into the Brisbane River estuary (~2000 tonnes nitrogen/yr) (Fig. 1). The eastern side of Moreton Bay receives low-nutrient oceanic water and few anthropogenic nutrients (Udy and Dennison, 1997).

δ^{15}N of Ambient Marine Plants

A variety of naturally occurring marine plants were collected throughout Moreton Bay and the estuarine regions of its catchment. Marine plant species collected included a seagrass (*Zostera capricorni*), attached macroalgae (*Gracilaria edulis*, *Catenella nipae*) and a mangrove (*Avicennia marina*). Following collection, plant material was oven dried and samples were oxidized in a Roboprep CN Biological Sample Converter (Dumas Combustion). The resultant N_2 was analysed by a continuous flow isotope ratio mass spectrometer (Europa Tracermass, Crewe, UK) for δ^{15}N isotopic signatures.

A strong west-east gradient of δ^{15}N signatures were identified in these naturally occurring marine plants (Fig. 2). Values were highest (~10‰) in proximity to sewage outfalls, values decreasing with increasing distance from the central regions of the western shore. Values around the mouths of the Caboolture and Logan Rivers were relatively low in comparison to values around the Brisbane and Pine Rivers mouths. Sewage nitrogen from upstream outfalls in the Caboolture and Logan Rivers appears to be dissipated by in-stream processes such as denitrification (loss as N_2 gas) and/or biotic uptake (e.g., bacterial, vegetative) resulting in low-δ^{15}N signatures downstream. There are large sewage outfalls located at the mouths of the Brisbane and Pine Rivers and marine plants near these sources had elevated δ^{15}N values. Marine plants collected from the oceanic influenced eastern portions of the bay had δ^{15}N signatures always less than 3.0‰.

δ^{15}N of Deployed Marine Plants

Utilising δ^{15}N signatures of naturally occurring marine plants is limited by their distribution. In regions receiving riverine inputs from urbanized catchments, growth of benthic marine vegetation is often restricted due to a number of variables, particularly the lack of light. Restricted marine plant distribution decreases the ability to map sewage plumes with adequate spatial resolution. In order to overcome restricted distributional ranges, samples of the red macroalgae, *Catenella nipae*, were incubated at approximately 100 sites along the western shore of Moreton Bay. At each site, macroalgae were housed in transparent, perforated chambers and suspended in the water column for 4 days at ~50% light (secchi disk/2) using a combination of buoy, rope and weights (secchi depth varied from 0.5–4 m) (Fig. 3). *C. nipae* (δ^{15}N ~ 2‰) was collected from the eastern side of Moreton Bay and deployed in situ in a radiating grid pattern adjacent to the four major river mouths entering Moreton Bay. Sites were selected using GIS software (ESRI Arcview 3.1) and located in the field using a differential Global Positioning System. Following deployment of macroalgae, samples were analysed for δ^{15}N using the same technique as used for the naturally occurring plants. This technique of macroalgal deployment was applied in September 1997 and February 1998. There was little rainfall in the region prior to and during both incubation periods.

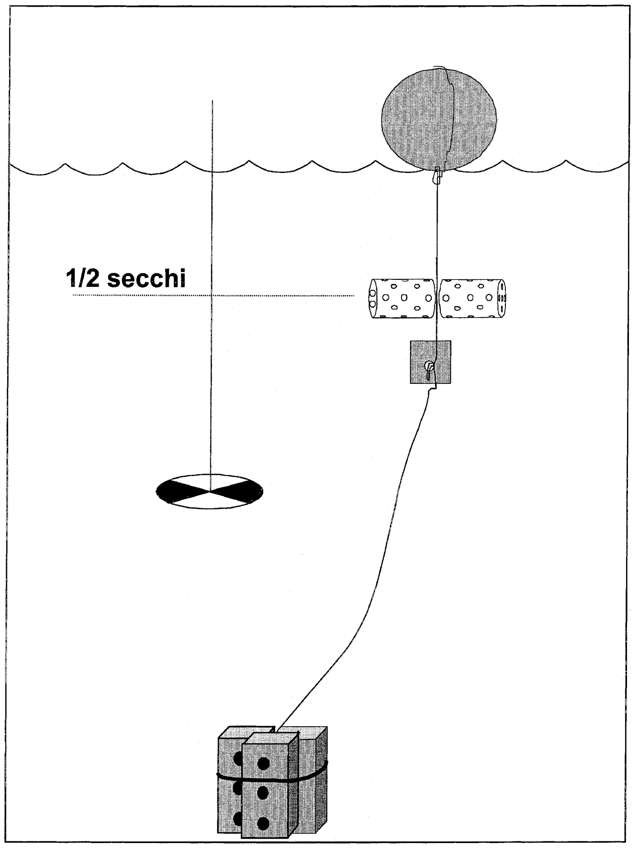
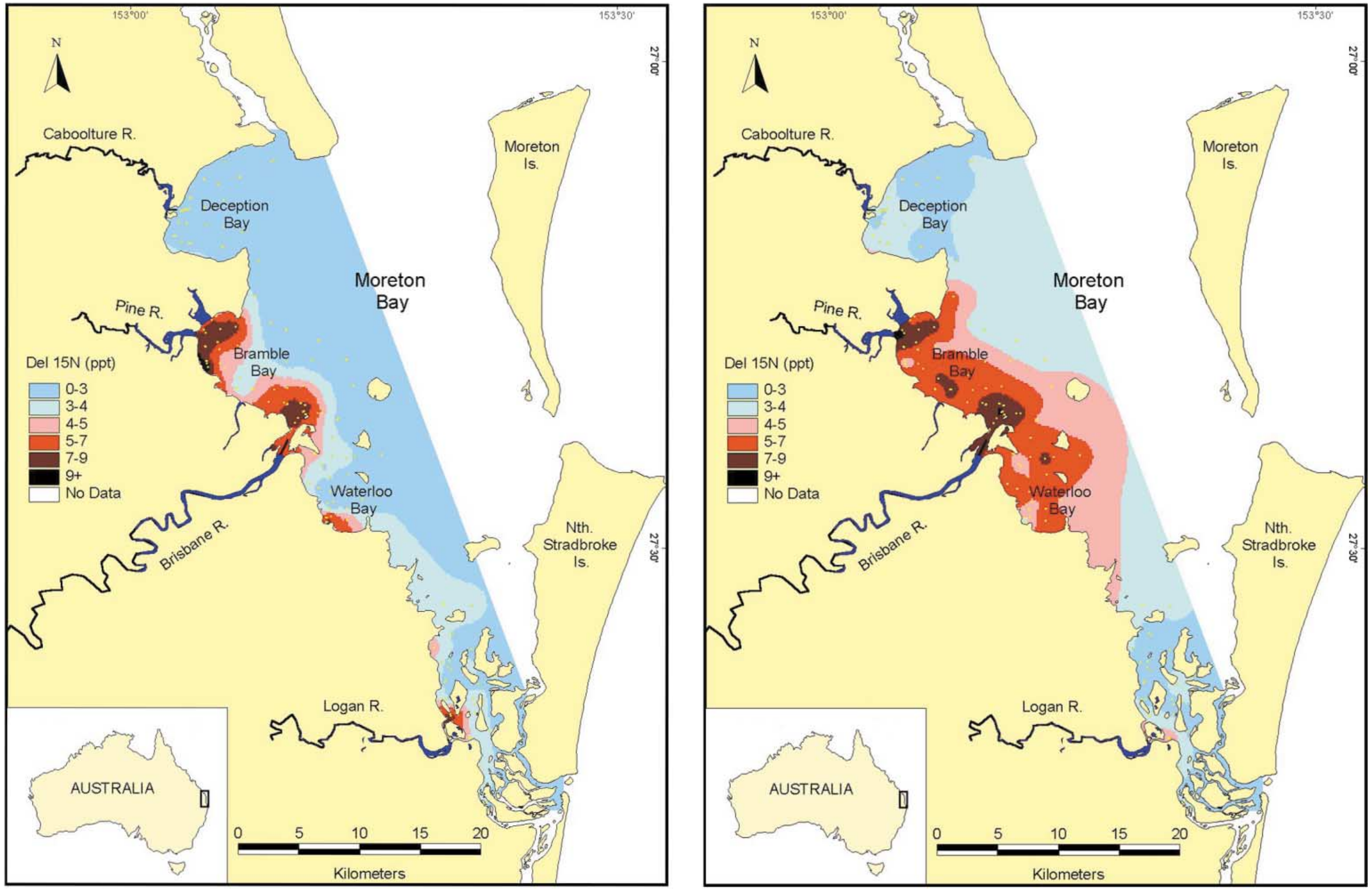


Fig. 3 Macroalgal deployment chambers.

Spatial analysis

Deployment sites were arranged radially around river mouths to provide a spatial resolution of sewage plumes. δ^{15}N values of the macroalgae following deployment were spatially interpolated using universal Kriging (Cressie, 1993), which also provided spatially structured estimates of uncertainty. Interpolations were back-transformed to the original scale. Contours of δ^{15}N were based on the logarithm of δ^{15}N, which was well described by a normal distribution, and which had stable variance. An exponential model was fitted to an empirical semi-variogram constructed from the residuals of a quadratic surface, fitted to the logarithm of δ^{15}N (to remove large-scale trends). δ^{15}N values were ranked into six categories, ranging from $< 3‰$ to $> 9‰$. Values above 3‰ indicate sewage-derived nitrogen was assimilated by the macroalgae. The contours of these six categories define the degree of biological influence of sewage nitrogen in the bay.

Two sewage plumes were evident emanating from the Brisbane and Pine Rivers (Fig. 4(a)). Macroalgal δ^{15}N increased from less than 3‰ in central-western Moreton Bay to greater than 9‰ in close proximity to



(a) (b)

Fig. 4 Spatial distribution of deployed macroalgal $\delta^{15}N$ values in September 1997 (a) and February 1998 (b). Macroalgae (*C. nipae*, Rhodophyte) was deployed at ~100 sites (yellow solid circles) in Moreton Bay, Australia.

sewage outfalls. $\delta^{15}N$ values of macroalgae were above 3‰ up to 5 km from these river mouths, indicating that the influence of sewage extended to this distance. Very little or no sewage signal was detected around the mouths of the Caboolture or Logan Rivers supporting the results measured with naturally occurring marine plants.

The distribution of $\delta^{15}N$ was found to be more widespread in February relative to September (Fig. 4(b)). Although two sewage plumes can still be discerned emanating from the Brisbane and Pine Rivers, the delineation is much less distinct. Whereas values approached control levels (< 3‰) at 5 km from the source in September, values were around 5‰ at this distance in February. $\delta^{15}N$ values were high in macroalgae deployed throughout Waterloo Bay, a feature not apparent in algae deployed in September. As with previous surveys, little or no sewage signal was detected around the Caboolture or Logan Rivers.

The variation in $\delta^{15}N$ values of the macroalgae between the two deployment periods may be related to a variety of biological and environmental factors. In either case, the biological effect of sewage-derived nitrogen is being measured. Although rainfall was similar between the two deployment periods, water column temperature increased by 5°C. This is likely to have increased

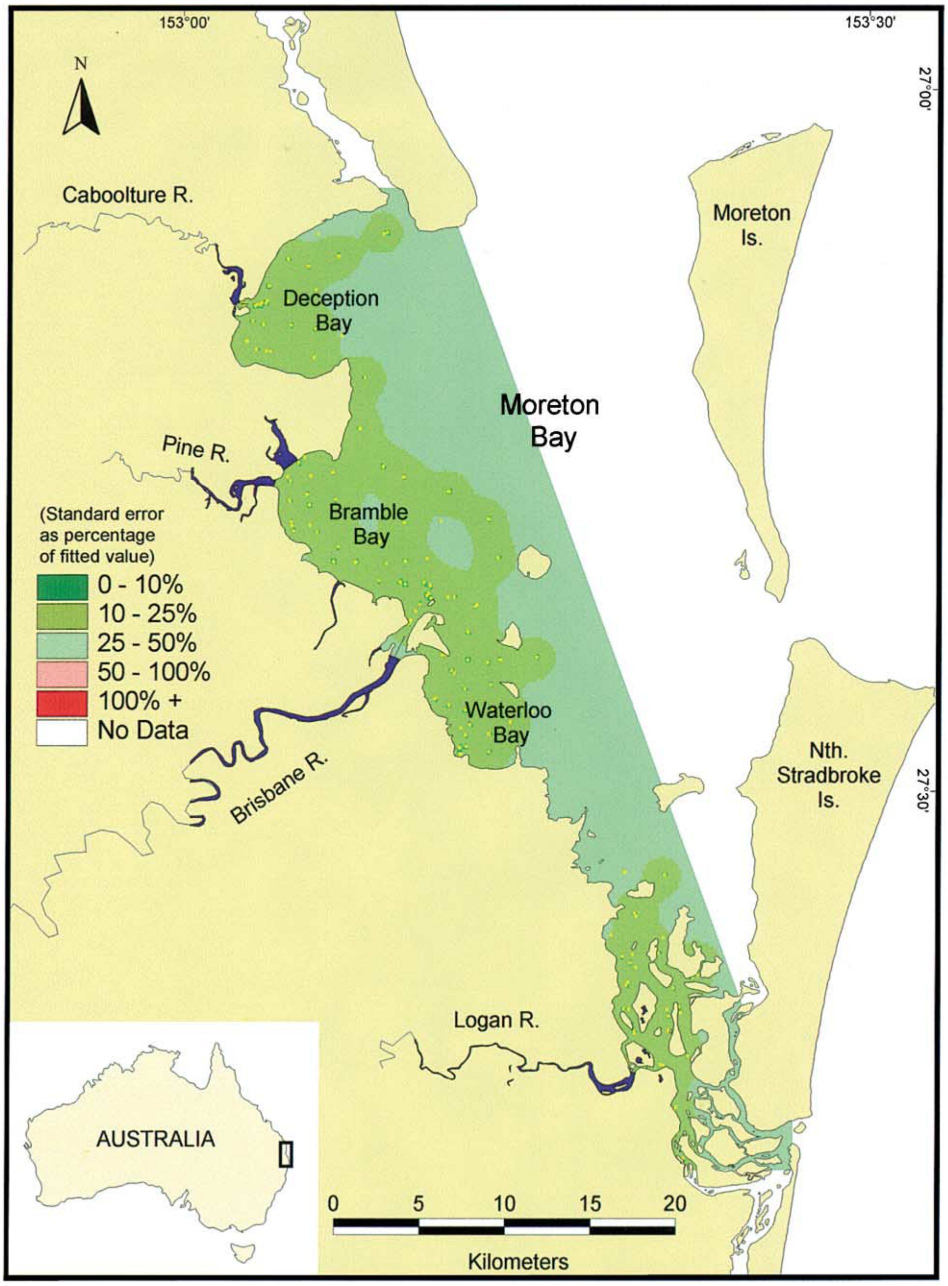


Fig. 5 Coefficient of variation map of interpolated $\delta^{15}N$ values for February.

macroalgal productivity in February, increasing algal uptake of nitrogen (Peckol, 1994). In addition, sediment microbial activity is enhanced with increased temperature (Jeffrey *et al.*, 1995), possibly increasing sediment mineralization of sewage-derived organic matter resulting in $^{15}NH_4^+$ fluxes into the water column available for algal uptake. These explanations may also be pertinent to the large response measured in Waterloo Bay in February. In addition, a hydrodynamic model of Moreton Bay predicts a south-westerly water flow in this region at this time of year. This seasonal circulation pattern may be responsible for driving sewage from Bramble Bay into Waterloo Bay (Dennison and Abal, 1999).

Statistical verification

An integral component of this technique was the estimation of uncertainty (coefficient of variation) of the interpolated data. Interpolation between samples is subject to error and maps must, therefore, contain estimates of uncertainty. Uncertainty was quantified as a coefficient of variation, obtained by expressing the standard error of prediction as a percentage of the interpolated value. The coefficient of variation provides a means of assessing the accuracy of the interpolated data. The coefficient of variation map produced for February indicates that the interpolated data between points is 75–90% accurate for the majority of the western shore (Fig. 5). Similar coefficient of variation patterns was observed for September (data not shown).

Applications

This technique of mapping biologically available sewage nitrogen has created a baseline for future water quality management and monitoring in the region. The results have highlighted the need for improved standards for sewage discharge. As a direct result of this study, six regional authorities have initiated improvements to sewage treatment facilities with emphasis on nitrogen removal and begun investigations and implementation of wastewater re-use schemes (MBCWQMST, 1999). This method of mapping sewage plumes has also been incorporated into a regional water quality monitoring program.

Summary

Previous techniques for detecting and mapping sewage effluent have provided information on various sewage components, however, in coastal ecosystems limited by nitrogen availability, the detection of nitrogen is of paramount importance. By using $\delta^{15}N$ signatures of marine plants, biologically available and therefore ecologically significant nitrogen can be detected and mapped in coastal ecosystems. The measurement of sewage impacts using marine plants has proven to be valuable for environmental management and monitoring of sewage nitrogen in Moreton Bay. We believe that this novel approach will lead to a reassessment of methods for detecting and mapping sewage impacts in many other coastal ecosystems.

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