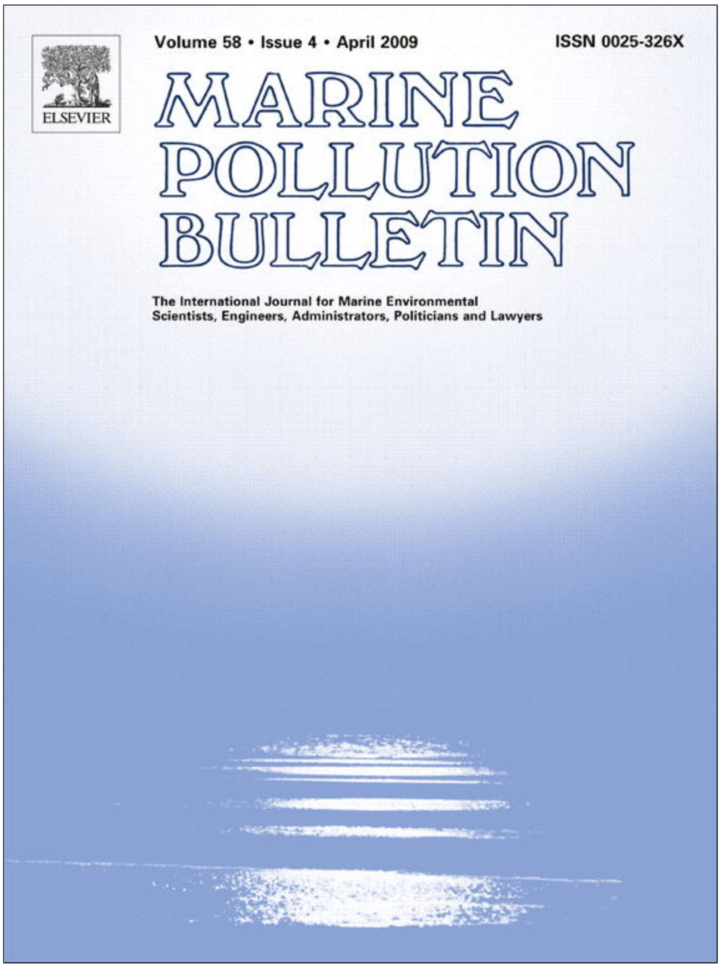
Volume 58 • Issue 4 • April 2009
ISSN 0025-326X
ELSEVIER
MARINE POLLUTION BULLETIN
The International Journal for Marine Environmental
Scientists, Engineers, Administrators, Politicians and Lawyers

Marine Pollution Bulletin 58 (2009) 573–580

Contents lists available at ScienceDirect

Marine Pollution Bulletin

journal homepage: www.elsevier.com/locate/marpolbul

Redistribution of sewage-nitrogen in estuarine food webs following sewage treatment upgrades

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ARTICLE INFO

Keywords:
Stable isotopes
Shore crabs
Mangroves
Algae

ABSTRACT

Stable nitrogen isotopes were used to assess the effects of wastewater treatment plant (WWTP) upgrades on the utilisation of sewage-N by estuarine biota in Moreton Bay, Australia. We measured $\delta^{15}N$ of filamentous algae, mangrove leaves and shore crabs at the Brisbane and Logan Rivers before and after scheduled WWTP upgrades, and at two reference rivers where WWTPs had been upgraded >4 years previously. The total N discharged into Brisbane River decreased by >80% after the upgrades had occurred, but N loads remained similar at Logan River despite the upgrade. In Brisbane River, $\delta^{15}N$ values of algae and crabs decreased and were comparable to the reference rivers within 1–2 years but no changes occurred at Logan River. The $\delta^{15}N$ of mangrove leaves remained elevated in all rivers, indicating that sewage-N remained a major source to mangroves either from residual WWTP discharges or from N accumulated in the sediments over many years.

1. Introduction

Sewage effluent is a major source of anthropogenic nitrogen to coastal systems. Since the growth of plants in marine systems is often limited by the availability of nitrogen (N), sewage-N is quickly assimilated by primary producers (Costanzo et al., 2001). As consumers graze on the primary producers, the sewage-N subsequently becomes incorporated into the food web (DeBruyn and Rasmussen, 2002; Piola et al., 2006).

Sewage-N is typically more enriched in ^{15}N than naturally occurring sources due to an enzymatic preference for ^{14}N by bacteria used to treat sewage in waste water treatment plants (WWTPs; Heaton, 1986). The tissues of primary producers that assimilate sewage-N, therefore, become enriched in ^{15}N. The enriched N signature is subsequently transferred to consumers with some fractionation in the $^{15}N/^{14}N$ ratio ($\delta^{15}N$) occurring between trophic levels. Thus the distinct ^{15}N signature in biota that derive much of their N from sewage can be used to determine the spatial extent of sewage plumes (Waldron et al., 2001; Costanzo et al., 2001; Gartner et al., 2002) and the degree to which sewage-N is incorporated into the food web (Spies et al., 1989; Hansson et al., 1997; DeBruyn and Rasmussen, 2002; Gaston et al., 2004).

Concerns about eutrophication have led to the upgrading of WWTPs to reduce the amount of sewage-N entering coastal waters. Reductions in the amount of sewage-N being incorporated into the food web can be measured by changes in the $\delta^{15}N$ of biota (Costanzo et al., 2005). The rate at which the isotopic signatures of primary producers respond to changes in the availability of sewage-N is likely to vary depending on factors such as their growth rates and the turnover time of elements in their tissues, and whether they source their N from the water column or the sediments. Rapidly growing species, such as filamentous algae, are likely to quickly adopt new isotopic signatures. There may be, however, a significant lag in the adoption of new isotopic signatures of more slowly growing species, such as mangroves or animals, which turn over N more slowly in their tissues (Gartner et al., 2002). Sediments also act as sinks for sewage-N through the burial of particulate organic matter including diatoms (Savage et al., 2004), and mangrove sediments in particular are recognised for their ability to accumulate and retain N (e.g. Rivera-Monroy and Twilley, 1996). Thus sewage-N is likely to persist in sediments for some time following upgrading of WWTPs. Changes to the water column, however, are likely to occur rapidly following reductions in the discharge of sewage-N. Primary producers such as mangroves, which derive the majority of their N from sediments via their roots (Alongi, 1996), may respond much more slowly to reductions in sewage-N than producers such as algae that source N from the water column (Wallentinus, 1984).

Moreton Bay is a coastal embayment in southeast Queensland, Australia. The city of Brisbane, with a population of approximately 1.5 million, is situated on the western side of the bay. Treated sewage effluent, which contributes 90% of point source N to Moreton Bay during dry periods (EHMP, 2004), is discharged from

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doi:10.1016/j.marpolbul.2008.11.016

numerous WWTPs situated within the tidal limits of several rivers that flow into the western bay. Concern about eutrophication in western Moreton Bay during the 1990s (Dennison and Abal, 1999) led to WWTPs in the Moreton Bay catchment being progressively upgraded to reduce N loads. Upgrades predominantly involved switching to or improving biological nutrient removal (BNR) systems. Reductions in the sewage-N entering Moreton Bay were mapped on four occasions between 1998 and 2003 by incubating the red alga, *Catenella nipae*, at approximately 100 sites arranged in a radiating grid pattern adjacent to the mouths of four rivers that discharge to the bay (Costanzo et al., 2005). A reduction in the spatial extent of sewage-N was observed, coincident with the upgrading of WWTPs. The study, however, examined incorporation of sewage-N into only one type of primary producer and the design of the study, which correlated changes in the distribution of $\delta^{15}N$ to changes in N loads, did not provide an unequivocal test of the effects of upgrading WWTPs on the incorporation of sewage-N into the food web. Despite this, a reduction in N loads from the WWTPs was the most parsimonious explanation for the observed trends.

In late 2005 two more WWTPs in the Moreton Bay catchment were scheduled for upgrades to their BNR systems. These were the Oxley WWTP (located on the Brisbane River) and the Loganholme WWTP (located on the Logan River). The upgrading of the Oxley and Loganholme WWTPs provided an opportunity to compare changes in the distribution of sewage-N in the biota of rivers that had WWTPs scheduled for upgrade, before and after the upgrades occurred, to those in rivers that had WWTPs that had been upgraded more than four years previously (i.e. control rivers). Thus the major objective of the study was to use a 'Before After Control Impact' experimental design to provide a robust test of the effectiveness of upgrading WWTPs on the incorporation of sewage-N into the estuarine food web. Specifically we aimed to:

1. track spatial changes in the distribution of sewage-N in estuarine biota,
2. determine the rate at which the $\delta^{15}N$ of algae and mangroves respond to changes in the availability of sewage-N, and
3. determine if reductions in sewage-N could be measured at trophic levels higher than primary producers.

2. Methods

2.1. Experimental design and variables studied

Four rivers with WWTPs that discharged into Moreton Bay were sampled. These were the Brisbane and Logan which had WWTPs that were scheduled for upgrade in late 2005 and the Pine and Caboolture which had WWTPs that had been upgraded in 2000 and 2001, respectively, and acted as controls. It was not possible to use rivers without WWTPs as controls, since the only rivers without WWTPs in the Moreton Bay catchment were very short (tidal limits at $\leqslant$ 13 km) and, therefore, were considered unsuitable for comparison against the Brisbane and Logan Rivers (tidal limits at 87 and 33 km, respectively). The WWTP outfalls were located at varying distances upstream but all were located within the tidal limits (Table 1). In all but the Pine River, the WWTPs were located in salinities of 10–17 and estuarine biota (e.g. mangroves, shore crabs) did not occur much further upstream of the WWTP.

2.2. Dissolved nitrogen loads

Dissolved inorganic nitrogen (NH_4^+ and NO_X) was measured monthly at multiple locations within each river from January 2004 to December 2005. Samples were collected as part of the Ecosystem Health Monitoring Program by the Queensland Environmental Protection Agency. Within each river, a single sample of water was collected at >5 locations spread between approximately 3 km upstream of the outfalls and the river mouths. Samples were filtered through 0.45 μm filters and frozen. NH_4^+ and NO_X were analysed using an automated LACHAT 8000QC flow injection analyser using photochemical methods.

2.3. Sampling of biota

Five locations spaced evenly between the outfalls (Location 1; the most upstream location) and the river mouths (Location 5) were selected at each river. At every location, three replicate samples of each variable were collected at each of two sites (separated by >50 m). Three variables were sampled: young leaves from the growing tips of the mangrove *Avicennia marina*, epiphytic filamentous algae on the pneumatophores of *A. marina*, and shore crabs (predominantly *Australoplax australis* but occasionally *Parasesarma erythrogramma*). Algae was selected as it grows rapidly and derives its nitrogen from the water column. The isotopic signature of algae, therefore, was likely to change rapidly in response to a change in availability of sewage-N. Mangroves derive their nutrients predominantly from the sediments and grow more slowly and, therefore, were predicted to respond to the decreased sewage-N more slowly than the algae. Shore crabs, which feed on particulate organic particles in the mud, were sampled to determine whether changes in N loads could be detected at trophic levels higher than autotrophs. Each river was sampled in August and again in November/December during 2005 (i.e. immediately prior to the upgrades), 2006 and 2007. Samples were placed in individual plastic bags and frozen immediately.

Due to variation in distributions of biota, it was not possible to always sample all taxa at each location. Shore crabs were never present at Location 1 (i.e. at the outfall) in the Caboolture River. The species of shore crabs present also varied among other locations. Where possible *Australoplax australis* was sampled but in the absence of this species, *Parasesarma erythrogramma* was collected. Algae were absent from the pneumatophores of *A. marina* at Location 1 in the Logan River on three of the six times sampled and on one occasion at Location 1 in the Caboolture River.

2.4. Stable isotope analyses

Algae and mangrove leaves were washed with filtered seawater to remove particulate matter, and muscle tissue was dissected from the legs of the crabs. All samples were dried to constant weight at 60 °C before being ground to a powder and weighed into

Table 1
Characteristics of the rivers and the annual N loads discharged prior to the upgrades and after the upgrades (2007) from the major WWTPs in the Brisbane, Logan, Caboolture and Pine Rivers.

River	Distance of outfall from river mouth	Salinity at outfall	Tidal limit	Annual N discharged 2002–2004 (*t*) (mean ± SE)	Total N discharged in 2007 (*t*)
Brisbane	47	11	87	303 ± 71	55
Logan	17	17	33	64 ± 8	77
Caboolture	19	11	20	38 ± 6	5
Pine	10	26	17	11 ± 5	27

tin capsules. Capsules were combusted on an Automated Nitrogen Analyser – Mass Spectrometer (Isoprime) at Griffith University, Queensland, Australia, and the products separated by gas chromatography to give a pulse of pure N_2 for analysis of total N and δ^{15}N. Isotopic ratios were converted to per mil (‰) values, calibrated against Atomic International Energy Agency standard N305A and atmospheric air, with a minimal standard error of 0.2‰ (Connolly et al., 2005). The following formula was used:

$$\delta^{15}\mathrm{N}_{\mathrm{sample}} = [({}^{15}\mathrm{N}/{}^{14}\mathrm{N}_{\mathrm{sample}} - {}^{15}\mathrm{N}/{}^{14}\mathrm{N}_{\mathrm{standard}})/{}^{15}\mathrm{N}/{}^{14}\mathrm{N}_{\mathrm{standard}}] \times 1000$$

2.5. Statistical analyses

Variations in dissolved inorganic nitrogen concentrations were interpreted graphically. Spatial and temporal variation in δ^{15}N of biota were analysed using ANOVAs. Prior to analyses the assumption of homogeneity of variances was tested using Cochran's test. Variances of all three variables were heterogeneous and could not be stabilised by transformation. Alpha, therefore, was reduced to 0.01 to reduce the likelihood of Type I error (Underwood, 1997). In the Brisbane River, concentrations of NH_4^+ and NO_X at locations adjacent to the outfall decreased greatly during the study. At the Logan River, however, reductions were observed in concentrations of NH_4^+ only. Consequently, the nature of the upgrades of the WWTPs in these rivers differed and the rivers, therefore, were not considered to be replicates. The four rivers, therefore, were treated as separate entities in the analyses. The occasional absence of algae and crabs at some locations also created an unbalanced data set. Slightly different analyses were done, therefore, on each variable. Preliminary analyses indicated that there was minimal variation in isotopic signatures between sites within a location. The six samples, therefore, were pooled for all analyses. Mangroves and crabs were analysed using 4-way ANOVAs. The factors were year (3 levels), month (2 levels), river (3 or 4 levels) and location (5 levels). All factors were fixed and orthogonal. All four rivers were analysed for mangroves but only the Brisbane, Caboolture and Pine Rivers were analysed for crabs due to the persistent absence of crabs at Location 1 in the Caboolture River. Algae was analysed using a 3-way ANOVA with the factors year, river and

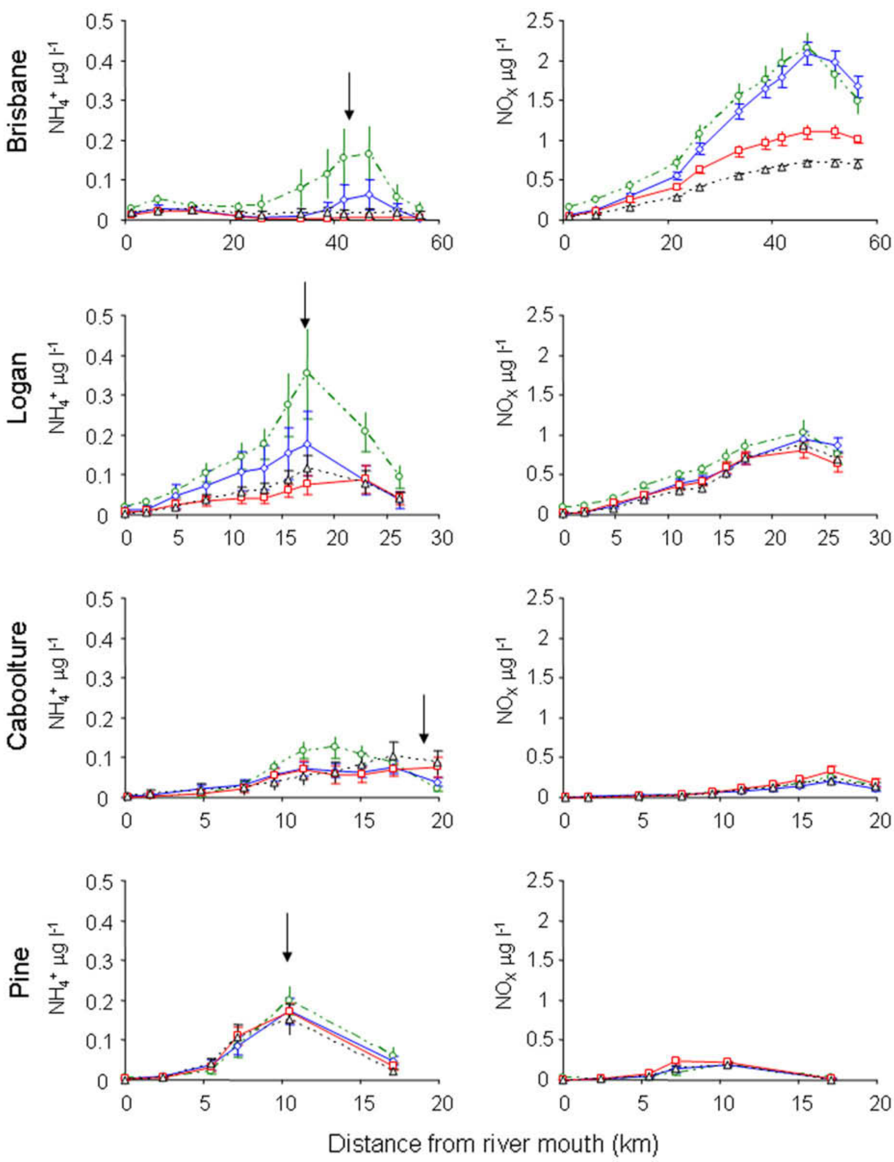

Fig. 1. Changes in average (±SE) annual concentrations of NH_4^+ (left) and NO_X (right) from the river mouths to upstream of the WWTP outfalls in the Brisbane, Logan, Caboolture and Pine Rivers during 2004 (– - ○ – -), 2005 (——◇——), 2006 (——□——) and 2007 (- - - △ - - -). *n* = 12. Arrows indicate the location of the outfall in each river.

location. Due to the inconsistent occurrence of algae at Locations 1 and 2 in the Logan River, only the Brisbane, Caboolture and Pine Rivers were analysed. Since algae was also absent on one occasion at Location 1 in the Caboolture River, six replicates were randomly selected from the Aug and Nov/Dec sampling periods. When significant differences were detected, post-hoc SNK tests were used to identify where the differences occurred. Although only a subset of the data were analysed for algae and crabs, data for all rivers were presented graphically.

3. Results

Overall, the total N discharged from the Oxley WWTP in the Brisbane River decreased from more than 300 t prior to the upgrades, to 55 t in 2007 (Table 1). N loads discharged from the Loganholme WWTP remained similar (67 vs 77 t). N loads discharged from the WWTPs in the reference rivers did vary (increased in the Pine but decreased in the Caboolture) but total loads were substantially smaller than those discharged from the Oxley and Loganholme WWTPs at the beginning of the study.

Table 2
Algae analysis. Results of 3-way ANOVA of $\delta^{15}N$ among years, rivers (Brisbane, Caboolture and Pine) and locations. DF = degrees of freedom; MS = mean square; F = F statistic. ns = non significant. Cochran's $C = 0.14^{**}$, $\alpha = 0.01$, $n = 6$.

Source	DF	MS	F
Year	2	174	42.1^{***}
River	2	81	19.5^{***}
Location	4	619	149.3^{***}
Y × R	4	25	6.1^{***}
Y × L	8	19	4.5^{***}
R × L	8	92	22.2^{***}
Y × R × L	16	19	4.5^{***}
Res	225	4	

** $P \leqslant 0.01$.
*** $P \leqslant 0.001$.

3.1. Dissolved inorganic nitrogen

In the two reference rivers, concentrations of NH_4^+ and NO_X were consistently low during all years, even adjacent to the outfalls (Fig. 1). Concentrations of NH_4^+ averaged less than 0.2 mg l^{-1} in the Caboolture and Pine Rivers. In the Brisbane River, prior to the upgrade concentrations of NH_4^+ were comparable to the reference rivers (approximately 0.2 mg l^{-1}), and as the upgrades came on-line, concentrations decreased progressively and by 2006 averaged less than 0.01 mg l^{-1} (Fig. 1). In the Logan River, prior to the upgrade NH_4^+ was elevated compared to the other three rivers (0.36 mg l^{-1}). Concentrations progressively decreased and by 2006 were comparable to those in the reference rivers. The pattern of change in NO_X concentrations was different from NH_4^+. In the Brisbane River, NO_X concentrations were initially 10 times greater than those in the reference rivers (approximately 2 mg l^{-1} in 2004; Fig. 1). Concentrations had halved by 2006 and were reduced to approximately 0.7 mg l^{-1} by 2007. There was no removal of NO_X, however, from the WWTP in the Logan River, with concentrations remaining consistently high (between 0.8 and 1.0 mg l^{-1}) throughout the study (Fig. 1).

3.2. Algae

There was considerable spatial and temporal variability in the $\delta^{15}N$ of algae, with a significant interaction occurring between year, river and location (Table 2). Post-hoc SNK tests revealed that trends were consistent with algae becoming depleted in ^{15}N in the Brisbane River following the upgrade of the WWTP. Indeed, at Location 1, $\delta^{15}N$ of algae decreased from 19.3‰ ± 1.4 in 2005 to 13.0‰ ± 0.6 in 2006/2007 (Fig. 2) Although the magnitude of the difference diminished downstream, $\delta^{15}N$ was significantly elevated in 2005 relative to 2006/07 at Locations 1–2. Variability among years was also detected at some locations in the reference rivers. At both rivers, however, $\delta^{15}N$ remained consistent among years at Location 1 (i.e. adjacent to the outfall), indicating that there was no change in the proportion of sewage-N being assimilated

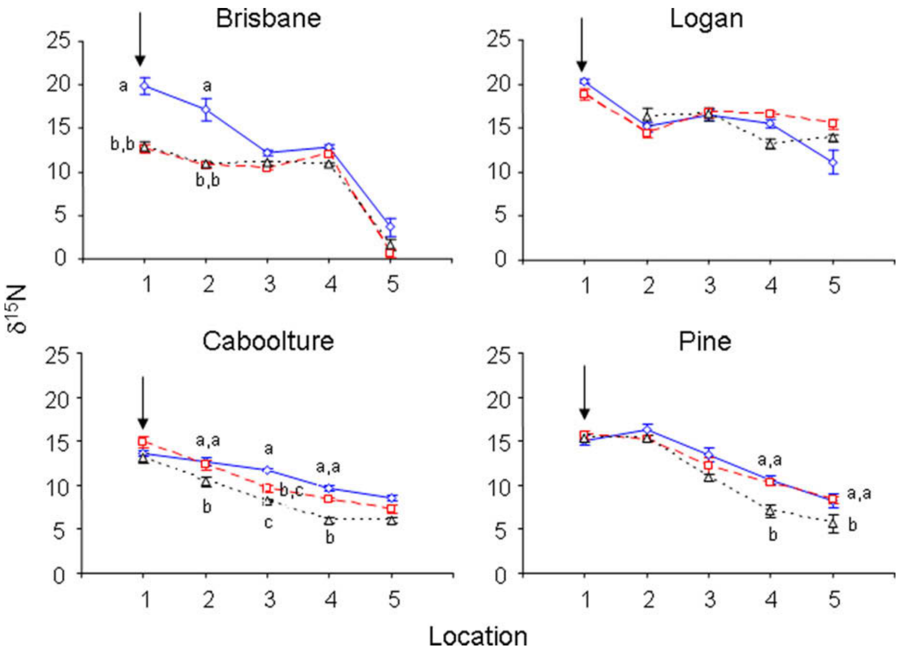

Fig. 2. Filamentous algae. Changes in the average (±SE) $\delta^{15}N$ values in the Brisbane, Logan, Caboolture and Pine Rivers during 2005 (—◇—), 2006 (– –□– –) and 2007 (- - -△- - -). $n = 6$. The Logan River was not analysed in the ANOVA. Arrows indicate the location of the outfall in each river. Letters indicate similarities (e.g. a,a; b,b; c,c) or differences (a–c) among years at each river and location based on SNK tests.

by algae during the study. The largest changes among years occurred at the downstream locations, indicating that the changes were caused by factors other than the amount of sewage-N entering the rivers. Although the Logan River was not included in the analysis due to the absence of algae at Location 1 during 2005 and 2007, there were no apparent trends in the data consistent with the upgrading of the WWTP. Indeed, there were no differences in δ^{15}N of algae among years at Location 2, suggesting that, even if there had been an effect at Location 1, the effect was localised.

3.3. Mangroves leaves

δ^{15}N of mangrove leaves exhibited considerable variability (Table 3) and although some trends were consistent with an effect of sewage-N, others were not. For example, prior to the upgrades in 2005 the δ^{15}N of mangrove leaves adjacent to the outfalls in the Brisbane and Logan Rivers (10.99‰) was greater than at the reference rivers (8.74‰) (Fig. 3). Also, the δ^{15}N of mangrove leaves at Location 1 in the Logan River decreased from 2005 to 2006/7. However, there was no significant decrease over time in δ^{15}N of mangrove leaves adjacent to the outfall in the Brisbane River, despite this river experiencing very large reductions in N-loads. δ^{15}N also increased from 2005 to 2006/7 at Location 1 at the Pine River (a reference river) and the magnitude of the increase (~2‰) was similar to the magnitude of the decrease (2.4‰) observed at Location 1 in the Logan River over the same period. A significant interaction between year, month, river and location was also detected (Table 3). Although there was variation between months sampled within each location, river and year, patterns of variation were not consistent with a seasonal trend. For example, differences between months were detected on only 12 out of the 60 possible year, month and location combinations. On 7 occasions mangroves were more enriched in August than December and on 5 occasions mangroves were more depleted in August than December. Overall, there was no compelling evidence to suggest that reduced N from the WWTPs loads caused a reduction in δ^{15}N of mangrove leaves in the Brisbane and Logan Rivers.

Table 3
Mangrove and crab analyses. Results of 4-way ANOVAs of δ^{15}N among years, months, rivers and locations. All four rivers were analysed for mangrove leaves but only the Brisbane, Logan and Pine Rivers were analysed for crabs. Cochran's $C = 0.08^{**}$ (mangroves) and $C = 0.10^{**}$ (crabs). $\alpha = 0.01$. $n = 6$. Details as per Table 2.

Source	Mangroves			Crabs		
	DF	MS	*F*	DF	MS	*F*
Year	2	22.2	7.3^{***}	2	152.2	123.1^{***}
Month	1	0.15	0.1^{ns}	1	131.0	106.0^{***}
River	3	154.2	50.4^{***}	2	198.6	160.6^{***}
Location	4	357.7	116.9^{***}	4	325.3	263.0^{***}
Y × M	2	17.1	5.6^{**}	2	218.7	176.9^{***}
Y × R	6	17.9	5.8^{***}	4	28.7	23.2^{***}
Y × L	8	12.2	4.0^{***}	8	13.9	11.2^{***}
M × R	3	13.5	4.4^{**}	2	6.52	5.3^{**}
M × L	4	19.2	6.3^{***}	4	12.8	10.3^{***}
R × L	12	70.9	23.2^{***}	8	32.3	26.1^{***}
Y × M × R	6	6.2	2.0^{ns}	4	45.6	36.8^{***}
Y × M × L	8	1.5	0.5^{ns}	8	13.3	10.8^{***}
Y × R × L	24	14.6	4.8^{***}	16	14.0	11.3^{***}
M × R × L	12	8.8	2.9^{***}	8	10.8	8.7^{***}
Y × M × R × L	24	6.0	2.0^{**}	16	10.6	8.5^{***}
Res	600	3.1		450	1.2	

3.4. Crabs

Variability among years at each river and location was consistent with an effect of the WWTP being upgraded in the Brisbane River (Table 3). In the Brisbane River, the δ^{15}N of crabs decreased progressively from 2005 to 2007 at Locations 1–3, indicating that the upgrade had an effect up to 15 km downstream of the outfall (Fig. 4). In the Logan River δ^{15}N was similar at Location 1 during 2005 and 2006 but decreased in 2007. There was, however, considerable variability in δ^{15}N among years at each location within the Logan River and trends were not consistent with the upgrading of the WWTP. There was no change at Location 1 in the Pine River and although there was variability among years at some locations,

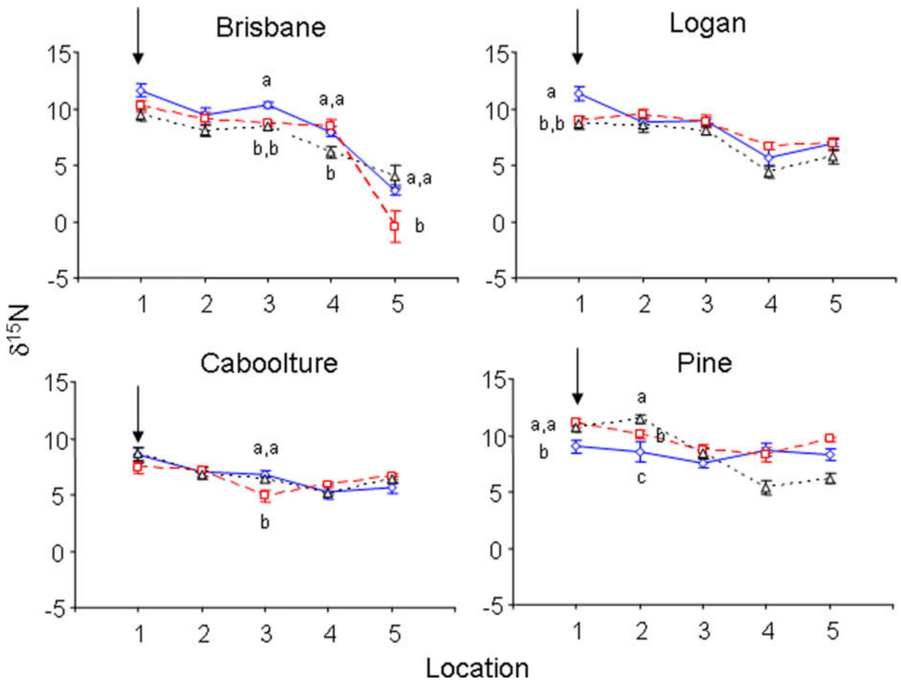

Fig. 3. Mangrove leaves. Changes in the average (±SE) δ^{15}N values in the Brisbane, Logan and Pine Rivers during 2005 (——◇——), 2006 (– –□– –) and 2007 (- - -△- - -). $n = 12$. Arrows indicate the location of the outfall in each river. SNK lettering as for Fig. 2.

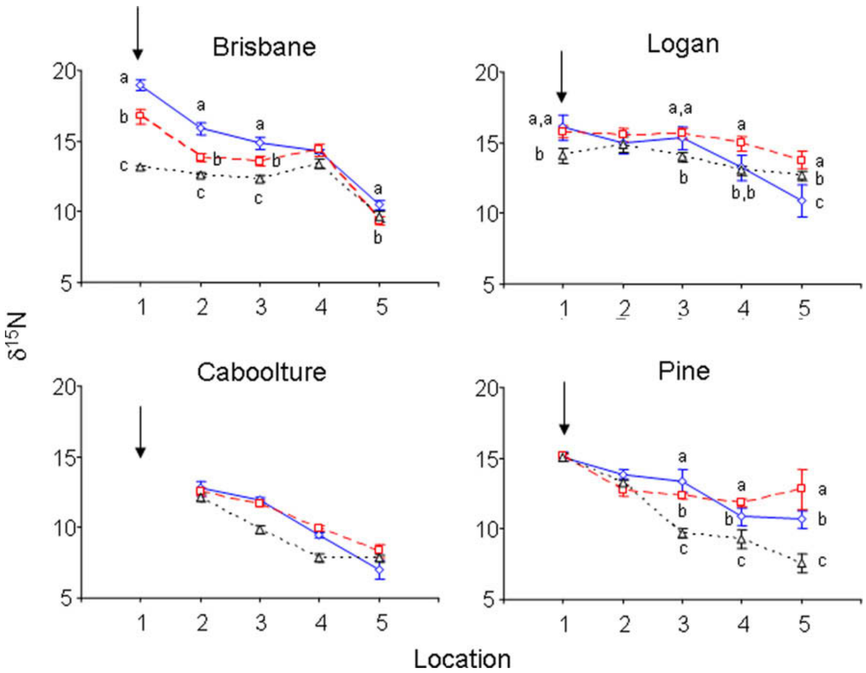

Fig. 4. Shore crabs. Changes in the average (±SE) $\delta^{15}N$ values in the Brisbane, Logan and Pine Rivers during 2005 (——◇——), 2006 (— —□— —) and 2007 (- - -△- - -). $n = 12$. Arrows indicate the location of the outfall in each river. SNK lettering as for Fig. 2.

the variability was not consistent with any change in the WWTP. As observed for mangroves, a significant interaction occurred between year, month, river and location (Table 3). Variability among months, however, was not consistent among places and years. On 18 of the 45 possible year, river and location combinations, crabs were more enriched in August than December. On 9 occasions they were more depleted and there was no difference between months on 18 occasions.

4. Discussion

The upgrading of the Oxley WWTP in the Brisbane River caused large reductions in $\delta^{15}N$ of algae and crabs. The effect was observed at the outfall and extended $\geqslant$10 km downstream. Such changes were not observed at the reference rivers, despite variability among years being detected at some locations. The magnitude of the changes at the Brisbane River for algae, in particular, greatly exceeded the variability among years at locations in the two reference rivers. Thus there was strong evidence that the changes in the $\delta^{15}N$ of algae and crabs in the Brisbane River resulted from the reduction in the discharge of sewage-N. In contrast, there was no evidence to suggest that upgrading of the Loganholme WWTP in the Logan River had any influence on the $\delta^{15}N$ of crabs. Although algae in the Logan River were not formally analysed and samples of algae were not available at Location 1 in 2005 or 2007, visual inspection of the graphs suggested that there were no changes in $\delta^{15}N$ of algae consistent with the upgrading of the WWTP. The lack of change in the isotopic signature of biota was consistent with the failure to reduce overall N-loads in the Logan River which may have resulted from the increasing residential population in the region that utilises the Loganholme WWTP.

The difference in the response of biota to the upgrading of the WWTPs in the Brisbane and Logan Rivers also reflected the differences in the types of upgrades that occurred at each WWTP. In both rivers, the largest decreases in NH_4^+ occurred before 2005 (i.e. prior to the study commencing) and after 2005 only relatively small decreases in concentrations of NH_4^+ were observed. In the Brisbane River, however, a large decrease in NO_X occurred between 2005 and 2006 but no change was observed in the Logan River. Large changes in $\delta^{15}N$ of algae and crabs were observed in the Brisbane but almost no changes were observed at the Logan. Although NH_4^+ is generally the preferred form of N, both algae (Dudley et al., 2001; Cohen and Fong, 2004) and mangroves (Naidoo, 1990), including *Avicennia marina* (Boto et al., 1985), can take up NO_X as well as NH_4^+. Thus it appears that the small change in NH_4^+ that occurred in the Brisbane and Logan Rivers had minimal influence on isotopic signatures and that the large changes in $\delta^{15}N$ observed in the Brisbane River were probably due to the decrease in NO_X, rather than NH_4^+.

In contrast to the changes observed in algae and crabs, upgrading of the WWTPs had no detectable effect on the $\delta^{15}N$ of mangrove leaves up to two years after the upgrades had been completed. The difference in the responses of the algae and mangroves largely agreed with our predictions that any reduction in $\delta^{15}N$ of mangroves would occur much more slowly than for algae. There are three major reasons why the response of mangroves would be slower. First, the growth rate and turnover time of nitrogen in mangroves is likely to be much slower than for filamentous algae. Second, mangroves source most of their N from the sediments via their roots (Alongi, 1996) and mangrove sediments can accumulate large quantities of N (Savage et al., 2004) and are likely to store N for extended periods. Third, unlike algae, mangroves recycle a proportion of their N internally since they resorb up to 64% of N from senescing leaves prior to abscission (Rao et al., 1994). Sampling over longer periods may be required, therefore, to determine if mangroves respond to the reduction in sewage-N. Interestingly, the $\delta^{15}N$ of mangrove leaves adjacent to the outfalls in the reference rivers (~7–11‰) were much more elevated than for leaves of the same species of mangrove sampled from areas distant from WWTP outfalls in other parts of Moreton Bay (~3‰; Melville and Connolly, 2003; Werry and Lee, 2005). The elevated $\delta^{15}N$ signatures indicated that the mangroves in the reference rivers were still utilising sewage-N despite the WWTPs in the reference rivers having been upgraded >4 years before the study commenced. To our knowledge, no studies have measured the duration for which sewage-N is retained in mangrove sediments. However, Savage et al. (2004) estimated that 5–11% of sewage-N was buried in sediments in Himmerfjärden, Sweden, and Holmes et al. (2000) estimated that more than 50% of total NO_3^- inputs were buried in sediments in the Parker River, Massachusetts. Consequently, sediments accumulate and store significant stocks of N. The elevated N signatures in the mangrove leaves of the Pine and Caboolture Rivers in the present study indicate that sewage-N probably remains in the sediments of these rivers more than four years after the WWTPs were upgraded.

The δ^{15}N values of shore crabs showed a clear response to sewage upgrades. Although both crab species feed on superficial sediments among the mangrove pneumatophores, their diets differ, as indicated by where they obtain their carbohydrates (Guest and Connolly, 2004). The crab sampled most often, *Australoplax tridentata*, tends to rely more on microalgae, whereas *Parasesarma erythrodactyla* is thought to rely more heavily on detritus. The protein source (and therefore N) for both species is yet to be confirmed, and possibly lies in a mixture of algae and detritus with some meiofaunal invertebrates. Given that the crab isotope values changed more rapidly and clearly in response to sewage treatment upgrades, it would appear that the crabs are drawing their N from a generalised N pool within the mangrove system rather than from mangroves specifically.

Upgrading of BNR systems often involves increased biological processing of sewage which can potentially inflate δ^{15}N of effluent (Costanzo et al., 2005). An increase in δ^{15}N of effluent would make reductions in the incorporation of sewage-N by biota more difficult to detect since the δ^{15}N of biota may have remained elevated despite overall reductions in N-loads. Changes in the δ^{15}N of the effluent were not measured but we consider it unlikely that potential changes in δ^{15}N confounded our results for two reasons. First, the lack of change in the δ^{15}N of biota in the Logan River was consistent with N-loads remaining relatively constant. Second, a large decrease in the δ^{15}N of algae and crabs was detected in the Brisbane River, even though an increase in the δ^{15}N of effluent would have made an effect more difficult to detect.

In coastal waters that are N-limited, the addition of sewage-N stimulates primary production. In some places subject to nutrient enrichment, increased secondary and higher-order production, including production of fish, has been observed (Colijn et al., 2002; Nixon and Buckley, 2002), and the enriched δ^{15}N values of sewage have been tracked through to fish in a local estuary (Schlacher et al., 2007). Thus, although the reduction of sewage-N entering Moreton Bay is likely to reduce issues associated with eutrophication, it may also reduce the overall productivity of the bay. The effects of nutrient enrichment (and, therefore, nutrient reduction) on food webs are complex. For example, variable nutrient concentrations can influence the efficiency with which energy is transferred between trophic levels (Kemp et al., 2001) and changes in stoichiometric ratios (e.g. N:Si) can influence whether phytoplankton communities are dominated by diatoms or dinoflagellates (Schöllhorn and Granéli, 1996). Studies examining how reductions in N loads affect the composition, functioning and productivity of food webs in Moreton Bay should be undertaken.

5. Conclusion

Upgrading of the Oxley WWTP in the Brisbane River reduced the amount of sewage-N being assimilated into the estuarine food web. The rate at which different components of the food web responded to reduced N loads, however, varied. Algae, which sources its N from the water column, showed a rapid reduction in the amount of sewage-N in its tissues. The amount of sewage-N utilised by crabs also declined, but more slowly than for algae. Mangroves showed no response to reduced N-loads, and δ^{15}N values of mangroves remained elevated in all estuaries. Sewage-N remains a major source for mangroves either via residual discharges from WWTP or from N accumulated in the sediments, suggesting that sewage-N might remain a significant source of N several years after WWTPs are upgraded.

Acknowledgements

We thank A. Boaden, S. Faggotter, K. Pardoe-Matthews, J. Webley, and the Ecosystem Health Monitoring Program team of the Queensland Environmental Protection Agency (M. Davidson, J. Ferris, J. Gruythusen, P. Hough, M. King, D. Renouf, M. Waller) for assistance with field and laboratory work. We also thank Rene Diocares for isotopic analyses and are grateful to the support provided by D. Rissik of the Queensland Environmental Protection Agency. The project was funded by a Griffith University Industry Collaborative Grant, the Queensland Environmental Protection Agency and Brisbane Water.

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