

# Assessing temporal and spatial trends in estuarine nutrient dynamics using a multi-species stable isotope approach



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## ABSTRACT

Coastal urbanisation can alter estuarine nutrient dynamics through the input of point-source and diffuse pollutants, and nutrient concentrations can be highly influenced by seasonal and episodic rainfall and river flow. Understanding of both the spatial and temporal variability of nutrient dynamics is therefore critical to managing these estuaries. This can be achieved by periodically analysing the stable isotopes a range of aquatic taxa with variable nutrient turnover rates, mobility and distribution within the estuary. In two subtropical urban estuaries with different land use patterns, we analysed the carbon and nitrogen stable isotopes of phytoplankton, shrimp, prawns and fish at various proximities to pollution sources in dry and wet seasons. The fast nutrient turnover rates and ubiquity of phytoplankton in the estuary resulted in stable isotopes varying over fine-scale spatial scales, particularly in relation to proximity to point-source pollution. The slower nutrient turnover rates and localised habitat use of prawns, resulted in stable isotopes varying over larger spatial (between pollution sources) and temporal (seasonal) scales. The much slower nutrient turnover rates and high mobility throughout the estuary of fish resulted in stable isotopes varying over very large-scale spatial scales (between estuaries). These results illustrate a wide range of spatial and temporal changes to estuarine nutrient dynamics in subtropical urban estuaries in relation to rainfall conditions and nutrient inputs. This research also highlights the application of stable isotopes in assessing estuarine trophodynamics, and provides direction on the types of organisms that should be used to assess different spatial and temporal trends.

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## 1. Introduction

Globally, estuaries are the foci of coastal urbanisation, which can disrupt the nutrient dynamics and ecology of these systems through direct habitat destruction, changes to hydrological flow (e.g. construction of dams), and input of both point-source and non-point-source pollutants (Cloern and Jassby, 2012; Lee et al., 2006). In particular, estuaries often receive significant nutrients from sewage treatment plants, storm water and industrial point sources, as well as more diffuse sources associated with urban and agricultural land uses (Lee and Olsen, 1985; Nixon et al., 1986; Vizzini et al., 2005). In excess, nutrients can have significant ecological impact in receiving environments, including facilitating phytoplankton blooms, eutrophication and hypoxia (Armon and Starosvetsky, 2015; Garnier et al., 2010; Romero et al., 2013).

In addition, distinct wet and dry periods in the subtropics and tropics can seasonally influence the trophodynamics of estuaries. Specifically, rainfall and river discharge can influence the relative contribution and availability of auto- and allochthonous production in estuaries (Cloern and Jassby, 2010). Understanding the spatial and temporal variability of nutrient dynamics is therefore critical, as well as challenging, to the management of estuaries, particularly in regions where rainfall and river flow are seasonal.

Stable isotope analyses, particularly for carbon and nitrogen, are useful tools for tracing the input and assimilation of nutrients in estuaries. Different sources of natural and anthropogenic nutrients have distinct elemental and isotopic composition. This, combined with relatively consistent fractionation between trophic levels ( $\sim 1\%$  for  $\delta^{13}\text{C}$  and  $\sim 3\text{--}4\%$  for  $\delta^{15}\text{N}$ ), mean that stable isotopes can be used to trace nutrients through food webs (Connolly et al., 2009; Fry, 2006), and identify the sources of nutrient inputs in estuaries (Connolly et al., 2013; Schlacher et al., 2007).

The different nutrient assimilation rates of organisms in an estuary allow investigations into trophodynamics over varying

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temporal scales. Phytoplankton assimilate nitrogen and carbon much more quickly per unit of biomass than do their consumers (Fry, 2006), therefore presenting a temporally responsive indicator for fluctuations in nutrient inputs. The stable isotopes of higher trophic level organisms, such as crustaceans and fish, represent the nutrients that are being assimilated from their prey (Fertig et al., 2010; Vizzini et al., 2005), and can provide a better time-integrated response to input of anthropogenic nutrients. Fish take up to several months to turnover their  $\delta^{13}\text{C}$  (Weidel et al., 2011), while prawns, having smaller body size, are expected to turnover  $\delta^{13}\text{C}$  within much shorter time frames. Prawns may therefore reflect changes in nutrient dynamics over shorter time frames (e.g. between seasons), whereas fish may provide a more temporally stable indication of the nutrient status of the system.

The mobility and distribution of different organisms allow investigations into estuarine trophodynamics at different spatial scales. Fish can be found over large salinity gradients, and are often highly mobile throughout an estuary (Schlacher et al., 2005). Their stable isotope signatures are therefore likely to represent a large spatial area. In contrast, prawns are generally less mobile and their stable isotope values will be more representative of the localised environment in which they are found. For example, juvenile penaeids are generally confined to the mangrove-lined creeks that run into main estuaries, for protection and nutrition during this early stage of their life-cycle (Meager et al., 2003). Further, although individual prawn species can be limited to particular salinity ranges, a variety of species sharing similar biology and ecology can be found along the entire range of estuarine habitats throughout the year. In subtropical estuaries, some prawn and shrimp species inhabit brackish and freshwater environments (e.g. the caridean *Macrobrachium* spp.) and others are restricted to higher salinities near the mouth (e.g. penaeids).

A multi-species approach encompassing seasonal wet and dry conditions may therefore provide the spatial-temporal resolution required for stable isotope values to accurately reflect the trophodynamics of subtropical estuaries. This approach should be especially useful in estuaries impacted by anthropogenic influences such as urbanisation, where additional nutrient sources may further complicate trophodynamics. This study investigated the stable isotope values of a suite of aquatic organisms with varying nutrient turnover rates, mobility and distribution to understand the influence of anthropogenic nutrient inputs on the trophodynamics of urban subtropical estuaries over different spatial and temporal scales. Phytoplankton, with very fast turnover rates and ubiquitous distribution throughout the estuary, may provide information at very fine temporal and spatial scales (within estuaries). Prawns, with moderate turnover rates and localised home ranges, may describe seasonal changes and broader scale spatial (i.e. between estuaries). And fish, with slow turnover rates and high mobility, may describe more time-integrated large-scale spatial differences. We demonstrate that this multi-species, spatio-temporal approach presents a comprehensive assessment of estuarine trophodynamics, and provides a framework for future investigations into trophodynamics in subtropical urban estuaries.

## 2. Methods

### 2.1. Study site

All sampling was conducted within the tidal limits of the Logan and Albert Rivers, southeast Queensland (Fig. 1). The Logan River discharges into southern Moreton Bay and is joined by the Albert River ~10 km upstream of the mouth.

The Logan and Albert catchments cover 3076 and 786 km<sup>2</sup>, respectively (EHMP, 2013), and land use in the upper catchments is

dominated by forests and grazing pastures with intermittent cropping (Fig. 2). In the estuarine regions of the catchments, where this study focuses, there is considerably more urban land use, with some agriculture (mainly sugarcane) and a number of prawn aquaculture facilities located near the mouth. The banks are lined by varying widths of mangrove forests dominated by *Avicennia marina* and *Aegiceras corniculatum*. There is no seagrass in the system due to the generally high turbidity. The lower reaches of the Logan estuary (downstream of the confluence) support commercial and recreational finfish and crustacean (mud crab, penaeid prawns) fisheries.

Using ArcGIS 10.1 (ESRI Inc, U.S.A.) and data compiled from the Queensland Land Use Mapping Project (DSITIA, 2013), the percent area of urbanisation was calculated to be 363 km<sup>2</sup> for the Logan catchment (12% of total catchment area), which was nearly six times larger than in the Albert (61 km<sup>2</sup>, 8% of total catchment area). There are two major sewage treatment plants (STPs) in these systems: the Loganholme STP that discharges into the Logan River approximately 6 km upstream of the confluence and the Beenleigh STP that discharges into the Albert River 3.5 km upstream of the confluence. The Loganholme STP discharges on average 50 ML of treated effluent into the Logan River each day (Jul 2010–Jun 2011), nearly four times the amount of effluent discharged into the Albert River by the Beenleigh STP (13 ML/day). Even if this is adjusted for the differences in mean river discharge volume (920 and 540 ML/day for the Logan and Albert rivers, respectively), the concentration of treated effluent in the Logan River is generally >2 times higher than in the Albert River.

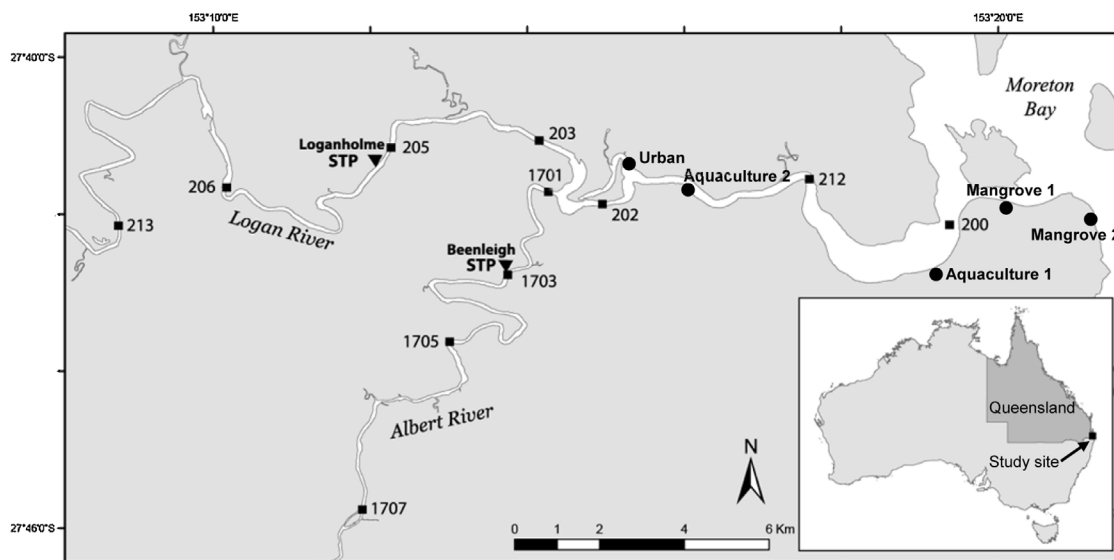
There were 11 collection sites within the Logan and Albert Rivers (Fig. 1). These sites corresponded to the sites regularly monitored for water quality parameters (e.g. temperature, turbidity, salinity, DO, pH, nitrogen, phosphorous, chlorophyll *a*) by the Ecosystem Health Monitoring Program (EHMP), a regional scheme that assesses the health of southeast Queensland waterways (EHMP, 2013), and included sites directly adjacent to sewage treatment plants at Loganholme (site 205) and Beenleigh (site 1703). Since the first EHMP report cards (2001), the Albert and Logan estuaries have scored D (poor) or F (fail), generally due to high turbidity, high nutrients (N and P) and low dissolved oxygen (DO). Over the study period, total N concentrations in the tidal reaches of the Logan and Albert Rivers ranged from 0.5–2.8 mg/L and 0.2–1.8 mg/L, respectively (EHMP, 2013), and were generally above the Queensland Water Quality Guidelines upper value for estuaries of 0.45 mg/L (DEHP, 2009).

Juvenile penaeid prawns were also collected from five tidal creeks that flowed into the main estuary through natural (mangrove) and anthropogenic (aquaculture and urban) land use areas (Fig. 1).

### 2.2. Sample collection

Phytoplankton, shrimp (*Macrobrachium australiense*) and fish (bony bream, *Nematalosa erebi*) were collected from the main estuary sampling sites during the dry (May and September 2011) and wet (February 2012) seasons. Phytoplankton was collected from each site by towing a plankton net (mesh size: 53  $\mu\text{m}$ , diameter: 30 cm, length: 85 cm) for 10 min at the surface. *M. australiense* and *N. erebi* were euthanised in an ice slurry immediately after collection and then frozen until processing for analysis.

In July 2011 and March 2012, juvenile penaeid prawns (*Metapenaeus* spp. and *Fenneropenaeus merguensis*, <3 mm carapace length) were collected from creeks in urban, aquaculture and natural mangrove areas of the Logan River (Fig. 1) using a beam trawl (0.5 m  $\times$  1 m) towed behind a boat, and light traps. Beam trawl runs began just upstream of the mouth of the creeks and were pulled upstream for 2–5 min against a falling tide. Light traps were placed



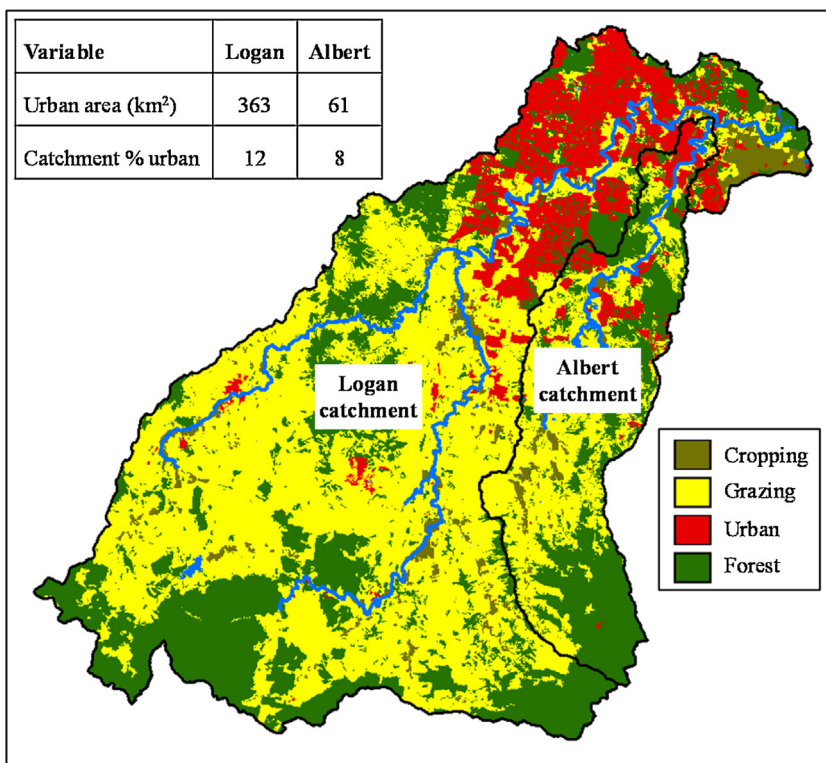
**Fig. 1.** Map of the tidal limit of the Logan-Albert estuary (southeast Queensland), including sample collection sites for phytoplankton and *Macrobrachium australiense* (■), and juvenile penaeids (●). Numbered sites are Ecosystem Health Monitoring Program (EHMP) sites where water quality information is collected monthly by the Queensland government.

inside the creeks (30–100 m upstream of the creek mouths), left overnight and collected the following morning.

### 2.3. Sample preparation and analysis

Phytoplankton was isolated from the seston collected in plankton tows using the sieve and spin technique (Hamilton et al., 2005). Briefly, the seston was initially sieved through a 250  $\mu\text{m}$  sieve (to remove large debris) and then through a 5  $\mu\text{m}$  mesh. A slurry was created from the >5  $\mu\text{m}$  seston with  $\sim 5$  mL of MilliQ water and

transferred to a 50 mL falcon tube. Approximately 40 mL of colloidal silica (density:  $1.21 \text{ g cm}^{-3}$ ; Ludox AM30, Sigma-Aldrich) was added and mixed thoroughly with the slurry. The silica/seston mixture was then centrifuged at 4400 rpm for 5 min and left to stand refrigerated for 24 h, allowing the lower density phytoplankton to settle on the surface of the silica. The phytoplankton layer was transferred to a clean 50 mL falcon tube and washed three times with MilliQ water to remove any remaining silica. The purified phytoplankton were transferred to a 5 mL tube, dried to a constant weight at 60 °C and homogenised.



**Fig. 2.** Map of the Logan-Albert catchment (southeast Queensland, Australia) with major land uses in each sub-catchment.

To test the purity of phytoplankton extracts, three replicate drops from each sample (before drying) were placed on a glass slide and examined under a compound microscope at 100× magnification. Five fields of view were randomly selected from each drop and a photo was taken. The number of phytoplankton and detritus particles was counted in each field of view and the mean purity (%) of phytoplankton was calculated for each drop. The purity (%) of each sample was calculated from the mean of all three drops, and an overall purity (%) of phytoplankton was calculated from the sample means.

#### 2.4. Stable isotope analysis

Phytoplankton and white muscle tissue of prawns and fish, free from skin, scales and gut tissues were dried to a constant weight at 60 °C and homogenised. The homogenised and dried phytoplankton (~10 mg) and animal (~1 mg) samples were accurately weighed into a tin capsule (5 mm × 8 mm), which was compressed into a pellet for stable isotope analysis. For analysis of carbon and nitrogen isotopes, dried samples were combusted in a Europa EA GSL elemental analyser coupled to a Hydra 2022 mass spectrometer (Sercon Ltd, UK). The ratios of  $^{15}\text{N}/^{14}\text{N}$  ( $\delta^{15}\text{N}$ ) and  $^{13}\text{C}/^{12}\text{C}$  ( $\delta^{13}\text{C}$ ) were expressed as the relative per mill (‰) difference between the sample and a conventional standards, air and Pee Dee belemnite limestone carbonate, respectively.

#### 2.5. Statistical analyses

The stable isotopes of phytoplankton were used to investigate fine-scale spatial (i.e. within estuary) and temporal (between seasons) influence of point-source pollution on estuarine trophodynamics, due to their ubiquity throughout each estuary and rapid stable isotope turnover rates. The  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values of phytoplankton were plotted against distance from the river mouth (a proxy for salinity), separately for each season and for the Logan and Albert Rivers. Due to the dominant flow of the Logan River, the sites downstream of the confluence were included with the four Logan River sites, and the Albert River was represented by the four sites upstream of the confluence. A line of best fit was applied to each Logan River data set only, as the four points of the Albert River were insufficient to establish meaningful relationships. In cases where the relationships between stable isotopes and distance to river mouth were linear, the slopes and elevations of regression lines were compared using analysis of covariance (ANCOVA). For each river, the slopes for the different seasons were compared by examining the significance of the interaction between season and distance from the mouth in an ANCOVA. In cases where the slopes were parallel (ANCOVA season × distance,  $p > 0.05$ ), a full factorial ANCOVA (season as the factor, phytoplankton isotope value as the dependent variable, distance from the mouth as the covariate) was performed to test differences in the elevation of the slopes between seasons. In cases where the ANCOVA was significant, least significant difference (LSD) post hoc analysis was used to identify which seasons were significantly different. No further analysis was done if the slopes were statistically different (ANCOVA season × distance,  $p < 0.05$ ).

Due to their slower stable isotope turnover rates and low mobility, *M. australiense* were used to investigate medium-scale spatial (within and between estuaries) and temporal (between seasons) influences of anthropogenic nutrient inputs into estuaries. Linear regressions, performed between stable isotope value and distance from the river mouth (for each season and for the Logan and Albert Rivers separately) indicated that there were no significant relationships. Subsequently, sites within each river were pooled for each season and a two-way ANOVA was conducted to test for differences between river (Logan or Albert) and season (May'11, Sep'11 or

Feb'12). If the interaction between season and river was significant, an ANOVA was performed for each season to investigate differences between rivers. If season was significant, least significant difference (LSD) post hoc analysis was performed to investigate which seasons were different ( $P < 0.05$ ).

The  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  stable isotopes of juvenile penaeid prawns that are resident in small creeks were used to investigate the inputs of anthropogenic nutrients (via these creeks) that have originated in and passed through different land use types. For sites where prawns were collected on both collection dates, the temporal variation in  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values was investigated with a Student's *t*-test. As there were no temporal differences in  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values at any of the collection sites (Student's *t*-test:  $p > 0.05$ ), mean ( $\pm$ SE)  $\delta^{13}\text{C}$  was plotted against mean ( $\pm$ SE)  $\delta^{15}\text{N}$  and a Student's *t*-test was used to investigate differences in carbon and nitrogen between anthropogenic (aquaculture and urban) and natural (mangroves and estuary) creeks.

Bony bream, having the slowest turnover rates and highest mobility, were used to test for large-scale spatial differences in trophodynamics, comparing the more urbanised Logan River to the less urbanised Albert. Due to insufficient replication of fish collected over all seasons, investigations into temporal variations of bony bream stable isotopes were not possible. Fish from all seasons were therefore pooled into Logan River and Albert River locations. The  $\delta^{13}\text{C}$  values were plotted against  $\delta^{15}\text{N}$  and a Student's *t*-test was used to investigate differences  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values of bony bream collected from the two rivers.

### 3. Results

The stable isotopes values of phytoplankton, prawns, shrimp and fish collected from the Logan-Albert estuary varied over different spatial and temporal scales. Stable isotopes of phytoplankton (fast nutrient turnover rates and ubiquitous in the estuary) varied in relation to point source pollutants and were diluted during the high flow wet season, identifying small scale spatial (within estuary) and temporal (between seasons) changes to estuarine trophodynamics. Stable isotopes of prawns and shrimp (medium turnover rates and localised distributions) also varied between seasons, and between different pollution sources, both within and between estuaries. The stable isotopes of fish (slow nutrient turnover rates and ubiquity in estuaries) varied between estuaries only, with no temporal variability. These results provide a comprehensive assessment of the spatial and temporal trophodynamics of the Logan-Albert estuary, and more generally, illustrate the different spatial and temporal resolution of these aquatic taxa in assessing estuarine trophodynamics (Table 1).

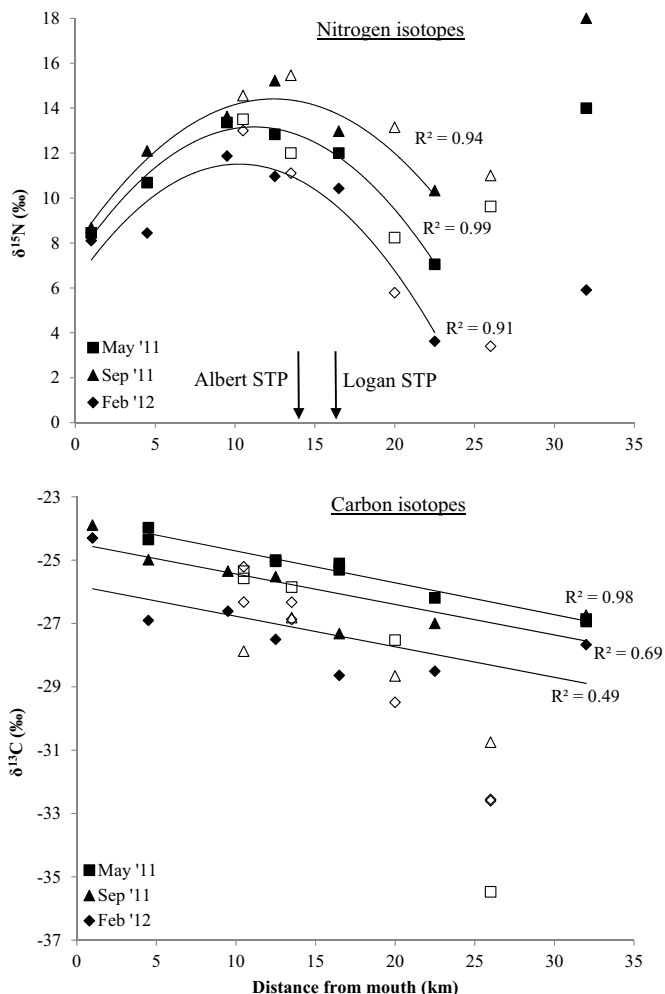
#### 3.1. Phytoplankton

The purity of the isolated phytoplankton samples was very high ( $89.8 \pm 4.3\%$ ), indicating very little detritus was contaminating the purified phytoplankton samples. Phytoplankton  $\delta^{15}\text{N}$  values showed distinct spatial and temporal patterns in relation to the STPs and season in both the Logan and Albert Rivers (Fig. 3a). Highest  $\delta^{15}\text{N}$  values were recorded in phytoplankton collected from the sites just downstream of the STPs and decreased with distance from this site both upstream and downstream. Only at the very upstream sites was this relationship interrupted, with very high  $\delta^{15}\text{N}$  observed again. Phytoplankton  $\delta^{15}\text{N}$  values at the Logan estuary mouth were consistently ~8‰, with minimal seasonal differences. However, seasonal differences were evident upstream of the mouth, with September values (end of dry season) consistently the highest and February values (wet season) consistently the lowest.



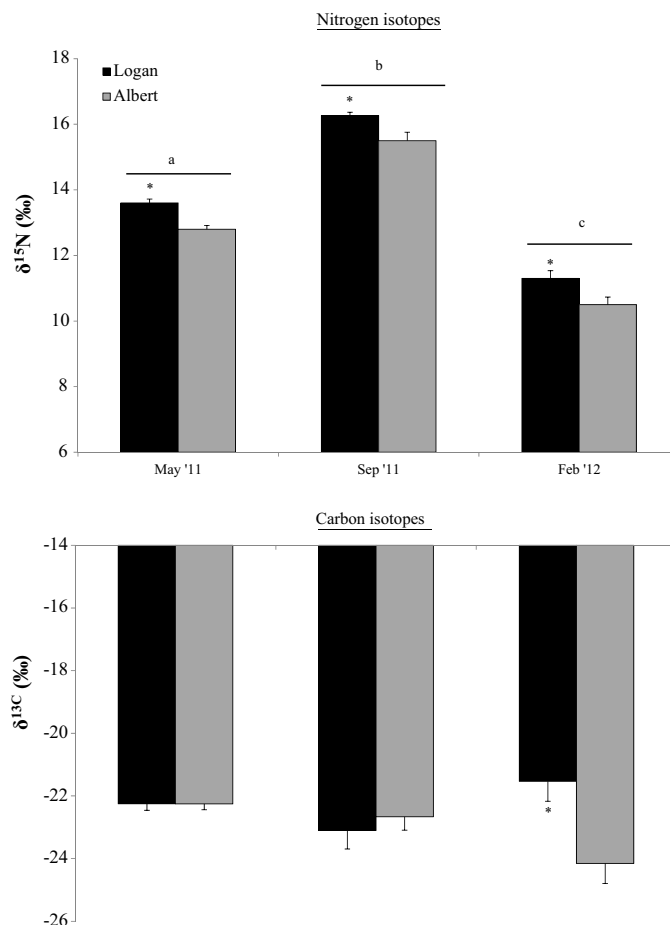
**Table 1**  
The stable isotope turnover rates and distribution within the estuary of different organisms, including how these characteristics influence the spatial and temporal resolution of these organisms in assessing estuarine trophodynamics.

Organism	Stable isotope turnover rate	Distribution	Spatial resolution	Temporal resolution
Phytoplankton	~Days	Ubiquitous	Within estuaries	Seasonal
<i>Macrobrachium australiense</i>	~Weeks	Localised	Between estuaries	Seasonal
Juvenile penaeids	~Weeks	Localised	Within estuaries	Yearly
Bony bream	~Months	Ubiquitous	Between estuaries	Yearly



**Fig. 3.** Relationship between the  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values of phytoplankton and distance from the mouth of the Logan (filled shapes) and Albert (open shapes) Rivers. Arrows indicate location of the Loganholme (Logan River) and Beenleigh (Albert River) sewage treatment plants. Samples collected on a flooding tide within three hours of the high in May (squares) and September (triangles) of 2011 and February 2012 (diamonds). Trendlines for Logan River data only due to small samples size for the Albert River.

Similar to the  $\delta^{15}\text{N}$  values, there was minimal seasonal variation in  $\delta^{13}\text{C}$  values of phytoplankton at the mouth of the estuary ( $\sim 24\text{‰}$ ) during both high- (Feb 2012) and low-flow (May and Sep 2011) seasons (Fig. 3b). However, there were linear relationships between  $\delta^{13}\text{C}$  and distance from the mouth in the Logan River, and there were different spatial and temporal trends between the Logan and Albert Rivers. In the Logan River,  $\delta^{13}\text{C}$  of phytoplankton were only slightly more depleted ( $\sim 3\text{--}4\text{‰}$ ) in the upper river sites and the slopes of the relationships between  $\delta^{13}\text{C}$  values and distance from the mouth were consistent over seasons (ANCOVA:  $F_{2,18} = 0.044$ ,  $p > 0.05$ ). However, there was a seasonal difference in this relationship (ANCOVA:  $F_{2,20} = 12.98$ ,  $p < 0.05$ ), with the  $\delta^{13}\text{C}$  of phytoplankton in the high-flow season (February 2012)

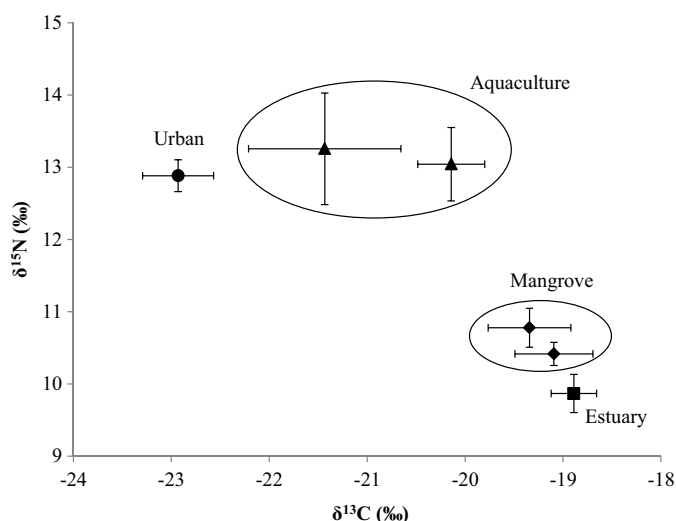


**Fig. 4.**  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values (mean, SE) of *Macrobrachium australiense* collected from the Logan and Albert Rivers. The  $\delta^{15}\text{N}$  of *M. australiense* collected from the Logan River were always significantly more enriched than those from the Albert River (\*; ANOVA:  $p < 0.05$ ) and there were also significant seasonal differences (a, b and c are significantly different; LSD:  $p < 0.05$ ). Only in February were the  $\delta^{13}\text{C}$  values significantly different (\*; ANOVA:  $p < 0.05$ ).

significantly more depleted (by  $\sim 2\text{‰}$ ) than in the low-flow seasons (LSD:  $p < 0.05$ ). In contrast, the  $\delta^{13}\text{C}$  of phytoplankton in the upper Albert River sites were much lower ( $\sim 6\text{--}12\text{‰}$ ) than at the mouth, with the most depleted value at the uppermost site (1707) in September 2011 ( $-35.5\text{‰}$ ) and the most enriched value in February 2012 ( $-26.8\text{‰}$ ).

### 3.2. Prawns and shrimps

There was a significant interaction between river and season for the  $\delta^{13}\text{C}$  values of *M. australiense* (2-way ANOVA for interaction:  $F_{2,30} = 5.07$ ,  $p < 0.05$ ), and only in February (wet season) were the  $\delta^{13}\text{C}$  values more depleted in the Albert River (Fig. 4; ANOVA:  $F_{1,11} = 8.32$ ,  $p < 0.05$ ). There was no significant interaction between season and river for the  $\delta^{15}\text{N}$  of *M. australiense* (2-way ANOVA for interaction:  $F_{2,30} = 0.37$ ,  $p > 0.05$ ). However, the  $\delta^{15}\text{N}$  values were



**Fig. 5.**  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values (mean, SE) of all juvenile penaeid prawns (*Fenneropenaeus merguensis* and *Metapenaeus* spp.) collected from urban, aquaculture and natural (mangrove lined) creeks, and from the main estuary in July 2011 and March 2012. Prawns collected from anthropogenic sites (aquaculture and urban) had significantly more enriched  $\delta^{15}\text{N}$  and depleted  $\delta^{13}\text{C}$  values, compared to the natural mangrove sites and prawns collected from the estuary (Student's  $t$ -test:  $p < 0.05$ ).

significantly more enriched in September 2011 and more depleted in February 2012, compared to May 2012 (Fig. 4; 2-way ANOVA for season:  $F_{2,30} = 308$ ,  $p < 0.05$ ; LSD:  $p < 0.05$ ). In addition, *M. australiense* collected from the Albert River had significantly lower  $\delta^{15}\text{N}$  values compared to those from the Logan River in all seasons (Fig. 4; 2-way ANOVA for river:  $F_{1,30} = 32.3$ ,  $p < 0.05$ ).

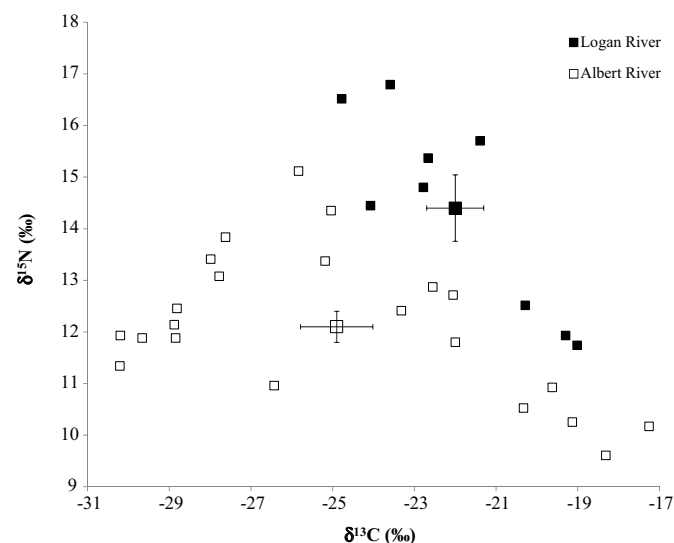
The  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values of juvenile penaeids collected from tidal creeks were significantly different, with prawns collected from anthropogenic sites (urban and aquaculture) having significantly more enriched  $\delta^{15}\text{N}$  and more depleted  $\delta^{13}\text{C}$  values, compared to the natural mangrove sites and prawns collected from the estuary (Fig. 5; Student's  $t$ -test:  $\delta^{13}\text{C}$ ,  $t_{1,42} = 6.58$ ,  $p < 0.05$ ;  $\delta^{15}\text{N}$ ,  $t_{1,42} = -9.41$ ,  $p < 0.05$ ). The  $\delta^{15}\text{N}$  values of all anthropogenic sites were consistent ( $\sim 13\text{‰}$ ), but the  $\delta^{13}\text{C}$  ranged from  $-20\text{‰}$  in one of the aquaculture creeks to  $-23\text{‰}$  in the urban creek.

### 3.3. Fish

Bony bream from the Logan River had significantly higher  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values compared to individuals from the Albert River (Fig. 6; Student's  $t$ -test:  $\delta^{13}\text{C}$ ,  $t_{1,29} = 1.97$ ,  $p < 0.05$ ;  $\delta^{15}\text{N}$ ,  $t_{1,29} = 3.67$ ,  $p < 0.05$ ). Interestingly, for the range of  $\delta^{13}\text{C}$  values occupied by fish from all sites, the  $\delta^{15}\text{N}$  values were always more enriched in the Logan River compared to the Albert River (Fig. 6).

## 4. Discussion

The stable isotopes of a range of organisms with different nutrient turnover rates, distribution and mobility presented here have provided a comprehensive assessment of the influence of point-source and diffuse-source nutrient inputs on estuarine trophodynamics over a range of temporal and spatial scales. The  $\delta^{15}\text{N}$  values of phytoplankton provided evidence of localised nitrogen inputs from sewage treatment plants that are diluted further from the source, as well as in the wet season when river flow is high. Phytoplankton  $\delta^{13}\text{C}$  values provided further evidence of dilution during the wet season and highlighted differences in carbon inputs between the two rivers. The stable isotopes of prawns and shrimps, which provide a more time-integrated indication of nutrient assimilation and remain relatively immobile in the system, indicated a



**Fig. 6.** The  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values (including the mean  $\pm$  SE) of all bony bream collected from the Logan and Albert Rivers. Bony bream collected in the Logan River had significantly higher  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values compared to those from the Albert River (Student's  $t$ -test:  $p < 0.05$ ).

larger input of sewage-derived nitrogen in the Logan River, which receives larger volumes of nitrogen-rich sewage effluent. In addition, the stable isotopes of juvenile prawns resident of tidal creeks indicated input of more diffuse sources of anthropogenic nutrient into estuaries. Finally, the stable isotopes of fish, which are highly mobile within a system and have slow nutrient turnover rates, provided evidence of varying inputs of both carbon and nitrogen between the two rivers.

### 4.1. Phytoplankton

The enriched phytoplankton  $\delta^{15}\text{N}$  values recorded throughout both rivers indicated a significant input of sewage-derived nitrogen that is assimilated into the base of estuarine food webs. Peaks in phytoplankton  $\delta^{15}\text{N}$  at sites just downstream of the Loganholme and Beenleigh STPs that decreased with distance from the source (both upstream and downstream) indicated that these STPs are a significant source of nitrogen contamination to this estuary. The return to more enriched  $\delta^{15}\text{N}$  values in the upper Logan and Albert River sites indicate another source of anthropogenic nitrogen in these areas, potentially from the seepage of septic tanks commonly used in these rural-urban areas. Phytoplankton  $\delta^{15}\text{N}$  values were generally lowest in the wet season (February 2012), indicating that the sewage-nitrogen signal diminishes due to dilution in these high river flow periods. In the dry seasons (September and May), tidal movement can transport STP nitrogen further upstream in the system resulting in the higher phytoplankton  $\delta^{15}\text{N}$  values observed at upstream sites. Interestingly, this seasonal pattern was not observed at the mouth of the estuary, where phytoplankton  $\delta^{15}\text{N}$  values were relatively constant over time. This may reflect a dominant marine influence at this site that does not change temporally. Support for this explanation comes from records indicating that even after high rainfall events, the mouth quickly returns to marine salinities (EHMP, 2013). These patterns in  $\delta^{15}\text{N}$  indicate that phytoplankton is a useful indicator of nitrogen pollution in estuaries, able to detect changes over relatively small spatial and temporal scales.

Interestingly, there were minimal differences in the  $\delta^{15}\text{N}$  values of phytoplankton between the Logan and Albert rivers in all seasons, despite the Logan River receiving more STP effluent with higher concentrations of nitrogen than the Albert River. The

Loganholme and Beenleigh STP effluents contain total nitrogen (TN) concentrations of ~6 mg/L and 3 mg/L, respectively. When effluent discharge rates and TN concentrations are adjusted for the differences in mean river discharge volumes over the study period (920 and 540 ML/day for the Logan and Albert Rivers, respectively), the concentration of sewage-derived nitrogen entering the Logan River is ~4 times more than in the Albert River. These differences were not reflected in the  $\delta^{15}\text{N}$  of phytoplankton in this study. However, because phytoplankton turnover stable isotopes very quickly, these values essentially represent a snapshot of sewage discharge in each system. A more time-integrated sample organism (e.g. prawns, see below) may better reflect the uptake of STP nitrogen in these estuaries.

The patterns in phytoplankton  $\delta^{15}\text{N}$  reported here are supported by Pitt et al. (2009) who found more enriched  $\delta^{15}\text{N}$  values for filamentous algae, mangrove leaves and shore crabs closer to the STP point sources in southeast Queensland estuaries. However, the phytoplankton  $\delta^{15}\text{N}$  values recorded at the mouth of the Logan River (~8‰) were slightly more enriched than those reported at the same location in 2003 using macroalgae (*Catenella nipae*) deployed in situ (Costanzo et al., 2005). This may be due to increased contribution of urban N to the total N pool of these rivers in recent times, as urbanisation of this region continues.

The  $\delta^{13}\text{C}$  of phytoplankton also showed a relationship with distance from the mouth (salinity) in the Logan and Albert rivers. Phytoplankton  $\delta^{13}\text{C}$  in the Albert River followed patterns expected from terrestrial vegetation-dominated systems: the  $\delta^{13}\text{C}$  values of phytoplankton in the upper Albert River sites (Fig. 3b; -35.5 to -30.8‰) were highly depleted, reflecting the strong influence from  $\text{C}_3$  plant sources (-20 to -37‰; Kohn, 2010). Due to the extensive removal of floodplain vegetation in this region of the Albert catchment, these  $\text{C}_3$ -influenced phytoplankton  $\delta^{13}\text{C}$  values are most likely a result of contribution from fringing mangroves (Maher et al., 2013). In contrast to the Albert River carbon dynamics, the narrow range over which  $\delta^{13}\text{C}$  values of Logan River phytoplankton varied along the river gradient indicates the dominant influence of a carbon source in this more urbanised system that is different to that in the Albert. This is supported by Sanderman et al. (2015) who found relatively consistent  $\delta^{13}\text{C}$  values (~-23 to -25‰) in the organic matter of a 132 cm deep sediment core (representing the last ~60 years) collected 14 km upstream of the confluence with the Albert. Only during infrequent extreme flooding events was there a significant wash-off of terrestrial  $\text{C}_3$  vegetation into the Logan River (Sanderman et al., 2015).

The phytoplankton  $\delta^{13}\text{C}$  values in the Albert River also indicate that the Logan River carbon dynamics is having some influence on the lower Albert River. The phytoplankton  $\delta^{13}\text{C}$  values at site 1701 (just upstream of the confluence) were very similar between seasons and within the range recorded in the Logan River. This indicates that this site is being influenced by the Logan River carbon dynamics during ebbing tides where water from the Logan flows upstream into the Albert. Upstream sites in the Albert River are further from this influence of the Logan River and phytoplankton values diverged towards the seasonal differences described above.

#### 4.2. Prawns and shrimp

The  $\delta^{15}\text{N}$  values of *M. australiense* displayed a similar seasonal pattern to the phytoplankton, with highest values in September (the dry season) and lowest in the wet season (February). Again, this is presumed to be due to the increased flow in the wet season that dilutes the  $\delta^{15}\text{N}$  in these systems. However, in contrast to the phytoplankton, a fine-scale (within river) spatial trend in  $\delta^{15}\text{N}$  values was not observed in relation to the location of the STPs for the *M. australiense*. This may reflect the fact that the  $\delta^{15}\text{N}$  in prawns is

integrated over a longer period of exposure to sewage-derived nitrogen (~8–10 weeks). However, this stronger time-integration can have advantages by being more representative of estuarine nutrient dynamics compared to the snapshot information in the phytoplankton. Indeed the significantly more enriched *M. australiense*  $\delta^{15}\text{N}$  values in the Logan River may better reflect the larger volumes of treated sewage with higher concentrations of total nitrogen being released from the Loganholme STP, compared to the Beenleigh STP. This further supports the need to include organisms with different stable isotope turnover rates when monitoring estuarine nitrogen pollution, to provide more comprehensive assessments of temporal and spatial trends in nutrient dynamics over different scales.

The  $\delta^{13}\text{C}$  values of the *M. australiense* also displayed similar patterns to the phytoplankton. The minimal seasonal differences in the  $\delta^{13}\text{C}$  values of *M. australiense* collected from the Logan River (~-22 to -23‰) further supports evidence of a dominant carbon source in this river that is relatively unaffected by rainfall and river flow. In addition, the significantly more depleted  $\delta^{13}\text{C}$  values in the *M. australiense* collected from Albert River, compared to the Logan River (although in this case, for February only), further indicates that the carbon dynamics of the Albert River reflects a less impacted, mangrove-dominated system.

The juvenile penaeids collected from the human impacted and natural creeks provided a further assessment of the input of anthropogenic nutrients into estuaries. The enrichment of the  $\delta^{15}\text{N}$  values of prawns collected from the urban and aquaculture impacted creeks were consistent with enrichment of producers (macroalgae and mangroves) previously reported in a nearby aquaculture creek (Jones et al., 2001). In addition, the low  $\delta^{13}\text{C}$  values in these anthropogenic creeks indicate alternative sources of carbon entering these food webs from these land use types. These results indicate that juvenile penaeid prawns are useful bioindicators of anthropogenic nutrients from more diffuse sources such as aquaculture facilities and golf courses. However, it should be noted that penaeids only utilise these creeks as juveniles, and due to their annual life cycle are only present in these creeks in significant numbers from January to July each year (Meager et al., 2003). Utilisation of these species for identifying inputs of anthropogenic nutrients to the estuaries is therefore limited to this time period.

#### 4.3. Fish

The  $\delta^{15}\text{N}$  values of bony bream were more enriched in the Logan River compared to the Albert (Fig. 6). The lower  $\delta^{15}\text{N}$  values in each river were within the ranges expected from the relationship between % urbanisation of catchment and fish  $\delta^{15}\text{N}$ , recently proposed by Morris et al. (2015), and reflect the higher levels of urbanisation in the Logan River catchment. However, the upper  $\delta^{15}\text{N}$  values in both rivers were considerably more enriched, and higher than the values expected from urbanisation alone. This may indicate the additional impact of sewage effluent discharge in these two rivers, as reflected by elevated phytoplankton  $\delta^{15}\text{N}$  values adjacent to the STP discharge points (see Fig. 3). And, similar to *M. australiense*, the bony bream also had significantly different  $\delta^{13}\text{C}$  values (Fig. 6), with bony bream from the Logan River more enriched (~-22‰), compared to the Albert River (~-25‰). This is consistent with the phytoplankton  $\delta^{13}\text{C}$  results, and further supports the dominant input of an enriched carbon source in the more heavily urbanised Logan River, and less impacted, mangrove-dominated carbon dynamics in the Albert River.

The spatial differences in both  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  observed in the bony bream (and not in the prawns or shrimp) also further support the need for multiple species to provide a more comprehensive assessment of estuarine nutrient dynamics. The stable isotopes of fish present a long-term time integrated representation of

nutrient inputs over large spatial scales. In fact, the movement and slow  $\delta^{15}\text{N}$  turnover rates of fish may be the reason Schlacher et al. (2005) found no seasonal differences in the  $\delta^{15}\text{N}$  of fish collected from the heavily urbanised Maroochy estuary, despite distinct seasonal changes to river flow. Fish on their own are therefore not sensitive enough to detect seasonal changes, but can be very useful in detecting large scale spatial changes in nutrient dynamics.

## 5. Conclusions

A comprehensive understanding of the impacts of point-source and non-point source nutrient inputs on estuarine trophodynamics requires assessment of spatial and temporal trends over a range of scales. Here we clearly illustrate how this can be accomplished via the combined use of organisms with different tissue turnover rates mobility and distribution, namely, phytoplankton, prawns, shrimps and fish. Phytoplankton, with fast turnover rates and ubiquity throughout the estuary can indicate fine-scale spatial (within estuary) variability in relation to point-sources (e.g. STPs), but provide only a temporal snapshot of nutrient conditions in the estuary. Prawns and shrimps have more time-integrated stable isotope values and can provide a more representative indication of seasonal changes and wider spatial scale (between estuaries and land use inputs) to estuarine nutrient dynamics. Finally, fish have very slow stable isotope turnover rates and travel extensively throughout estuaries, providing an even more time integrated indication of the nutrient dynamics of a system, allowing more comprehensive assessments over larger spatial scales (between estuaries). Combined use of species with different degrees of integration over temporal and spatial scales should prove particularly useful for unravelling the trophodynamics of seasonally and spatially variable ecosystems such as tropical and subtropical rivers, especially when anthropogenic influences such as discrete nutrient sources (e.g. point-source discharge from STPs) are present. Furthermore, knowledge of the spatial and temporal resolution of different aquatic taxa will allow more targeted and streamlined approaches to investigating specific aspects of estuarine trophodynamics in the future.

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