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δ^{15}N of estuarine fishes as a quantitative indicator of urbanization

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Abstract

Nitrogen stable isotope values (δ^{15}N) have commonly been used as a qualitative indicator of catchment urbanization in estuaries, but no quantitative relationship has to date been established between δ^{15}N and degree of urbanization. We sampled five species of common estuarine fish (*Mugil cephalus*, *Acanthopagrus australis, Silago ciliata, Gerres subfasciatus* and *Herklotsichthys castelnaui*) of different trophic levels from six estuaries in Southeast Queensland, Australia, to test if a quantitative relationship exists between δ^{15}N and urbanization in the catchment. Degree of urbanization (Urban %) in the catchment adjacent to the tidal estuary was measured using polygons constructed in Google Earth images, and verified using data from the detailed Queensland Land Use Mapping Project (QLUMP). Significant linear relationships exist between fish δ^{15}N and Urban % in most cases irrespective of the section of the estuary (upper, middle and lower) where the fish were caught, with no difference in slope and elevation between lines established for different sections. Inclusion of additional fish δ^{15}N data from other Australian estuaries in a combined multiple regression model adding trophic level as a predictor generated a significant relationship predicting increase in δ^{15}N values with increasing Urban % and trophic level. There is therefore a generic quantitative relationship between estuarine fish δ^{15}N values and degree of urbanization applicable to different fish species, geographic location or feeding habit. While the generality of this relationship needs to be tested further in other geographic locations, our findings potentially improve the applicability of fish δ^{15}N values as a quantitative indicator of urban influence in estuaries.

Key words: estuaries, fish, δ^{15}N, urbanization, trophic level, quantitative indicator, Google Earth

1. Introduction

Urbanization, the migration of people from rural to urban areas, is increasing globally, particularly in coastal areas surrounding major estuaries (Martinez et al., 2007). Urbanization can alter hydrological, sedimentation or nutrient dynamics regimes within estuaries, leading to structural modifications and reduced functionality of the ecosystem, causing an overall reduction in estuarine productivity (Dalrymple et al., 1992; Fulton et al., 1993; Whitfield and Harrison, 2008). Human population increase and the resultant demand for urban and agricultural land is now recognised as a major contributing factor to wetland loss and poor waterway quality in many coastal areas of the world (Lee et al., 2006; Vernberg et al., 1992).

Peri-urban estuarine ecosystems receive anthropogenic bioactive materials (e.g. bacteria/invasive species, drugs, nutrients), which can affect primary productivity, food web interactions and energy flow (Vanni et al., 2005). Introduction of excess nutrients (e.g. nitrogen and phosphorus) through municipal and agricultural wastes, pollutant run-off from impermeable surfaces and/or effluent input from wastewater treatment plants can change the nutrient dynamics of urban catchments and have flow-on effects to the trophic ecology of receiving estuaries (Carabel et al., 2006; Hansson et al., 1997; Hobson, 1999; Post, 2002; Schlacher et al., 2005). Nitrogen input from sewage treatment plants, for example, has been shown to alter population dynamics, change species assemblages and reduce more natural food sources such as seagrass (McClelland and Valiela, 1998; Schlacher et al., 2005). Shifts in organic matter source such as replacement of native seagrass by invasive algae could result in changes in the abundance and composition of the macrobenthos (Bishop and Kelaher, 2013; Taylor et al., 2010).

Growing concern over the impacts of urban development on estuaries has led to an increase in the demand for ecological information on which to base management plans and decisions (Attrill and Depledge, 1997; Whitfield and Elliott, 2002; Whitfield and Harrison, 2008). Water quality testing can provide important information on estuarine conditions (Attrill and Depledge, 1997), and, if performed regularly, can detect changes to ecosystem health over time. Water quality monitoring, however, only represents 'snap-shot' conditions of an estuarine system, without adequately detecting long-term, complex biological interactions and processes (Whitfield and Elliott, 2002; Whitfield and Harrison, 2008).

Bio-indicator organisms continually assimilate the environmental conditions of a system, allowing long-term time-integrated changes to nutrient dynamics to be investigated (Attrill and Depledge, 1997; Whitfield and Elliott, 2002; Whitfield and Harrison, 2008). Nitrogen stable isotopic ratios (δ^{15}N) are regularly used to indicate the trophic level of aquatic organisms (Cabana and Rasmussen, 1994; Fry, 1991; Layman et al., 2012; Post, 2002), with average enrichment of ~3-4 ‰ per trophic level (Faeth et al., 2005; Fry, 1991; McCutchan et al., 2003). In addition, δ^{15}N has also been established as a qualitative tracer for the assimilation of anthropogenic nitrogen sources, such as treated sewage effluent, which have elevated δ^{15}N values (Connolly, 2003; Davis et al., 2012; Hadwen et al., 2007; Mazumder et al., 2011; Post, 2002).

Quantitative relationships between the degree of urbanization and nitrogen isotopic ratios are, however, absent from the literature. Schlacher et al. (2005) demonstrated enrichment of the

^{15}N of fish over three estuaries with differing sewage inputs, but no attempt was made to quantify the general urbanization- δ^{15}N relationship. While δ^{15}N values are already incorporated into some routine environmental health monitoring programs (e.g. the Environmental Health Monitoring Program in Southeast Queensland, Australia), a generic quantitative relationship between δ^{15}N and the magnitude of urban influence on the biota, if it exists, would improve the usefulness of this indicator.

There is also a need to test the existence of a general quantitative relationship between urbanization and δ^{15}N in different locations along the tidal gradient of estuaries rather than the typical proximity-to-source approach. Locations further upstream receiving concentrated catchment run-off may be more impacted than the estuary, which receives significant oceanic influence and dilution (Hadwen et al., 2007; Piola et al., 2006; Schlacher et al., 2005). However, the mobility of potential important bio-indicators such as fish (Whitfield and Harrison, 2008) and the tidal action within estuaries may result in more even and widespread influence of estuarine nitrogen inputs (Hadwen et al., 2007; Loneragan and Bunn, 1999), and thus decreasing the usefulness of δ^{15}N as a quantitative indicator of urban influence.

In this study, we analyzed five species of estuarine fish at various trophic levels collected from six estuaries in catchments in Southeast Queensland, Australia, with urban development ranging from 8 to 26% to investigate the direct relationship between the degree of urbanization and δ^{15}N. A second component involved analysis of literature δ^{15}N values covering another seven estuaries in Australia spanning over between 0.1 to 59% urbanization in the lower catchment. Since δ^{15}N is known to vary according to the trophic level of animals, our model further attempted to address the two sources of influence simultaneously, with the aim of establishing a generic quantitative relationship incorporating these two predictors of the δ^{15}N of estuarine fish in per-urban estuaries in Australia.

2. Methods

2.1 Estimating % urbanization in catchments

2.1.1 Southeast Queensland estuaries

This study was conducted in six sub-tropical estuaries of Southeast Queensland (SEQ), Australia: Noosa, Maroochy, Pimpama, Coomera, Tallebudgera and Currumbin (Figure 1). These estuaries were selected due to the variations in urban land use in each catchment, providing a gradient of catchment urbanization (from ~7% to ~25%) over the six estuaries. The % urbanization of each estuary was estimated using Google Earth images (Google, 2013). Google Earth has been used as a geo-spatial tool (in conjunction with other GIS programs) for environmental monitoring and analysis (including land usage, native vegetation losses, population spread or disaster impacts), in many parts of the world (Chang et al., 2009; Clarke et al., 2010; Giri et al., 2011; Sato and Harp, 2009) and can provide current as well as historical information on global landscapes. Although arguably less accurate than direct estimates from GIS approaches, Google Earth imagery, due to its availability and coverage, allows for a quick and readily accessible database of urban development and land uses around each estuary for analysis.

Due to the concentration of urban developments in coastal areas and daily tidal flushing, Google Earth calculations of urban % were restricted to the lower catchment. Upper and lower limits of each catchment were defined as the tidal limit and mouth respectively, and lateral limits were defined by the lateral catchment boundaries. An image of each lower catchment was imported into Google Earth, and a polygon was traced around the boundaries to calculate the total area of each catchment. Within these lower catchment polygons, additional polygons were created around the urban or built up areas (residential, industrial, towns or cities). The urban % for each catchment was then calculated as sum area of urban polygons divided by the total catchment area (x 100).

To establish the accuracy of the Google Earth method, estimates of urban % for each of the six original estuaries were compared to Queensland Land Use Mapping Project (QLUMP) urban % calculations. Using ArcGIS (ESRI, USA), the percentage area of urbanization (urban %) for each catchment was calculated from QLUMP land use data (Queensland Department of Science, 2013): the sum of urban land use categories, namely ‘manufacturing and industrial’, ‘residential’, ‘services’, ‘transport and communication’, ‘utilities’ and ‘waste treatment and disposal’, for each catchment was calculated as a percentage (%) of the total catchment area. This is generally considered a more accurate measure of % urbanisation, but these data are only available for catchments within Queensland, Australia. Direct comparisons with other estuaries within within Australia or elsewhere are therefore not possible, limiting the application of the QLUMP approach.

Concordance in the Google Earth and QLUMP values was assessed using Pearson correlation. Urban % values estimated using QLUMP and the Google Earth method for the original six estuaries showed a significant correlation (Pearson r, $p = 0.036$, $r = 0.840$, $n = 6$) suggesting that the Google Earth method was an acceptable substitute of the more accurate but less widely available QLUMP method. To ensure consistency in the analysis, all reported Urban % data in this study are therefore based on the Google Earth method.

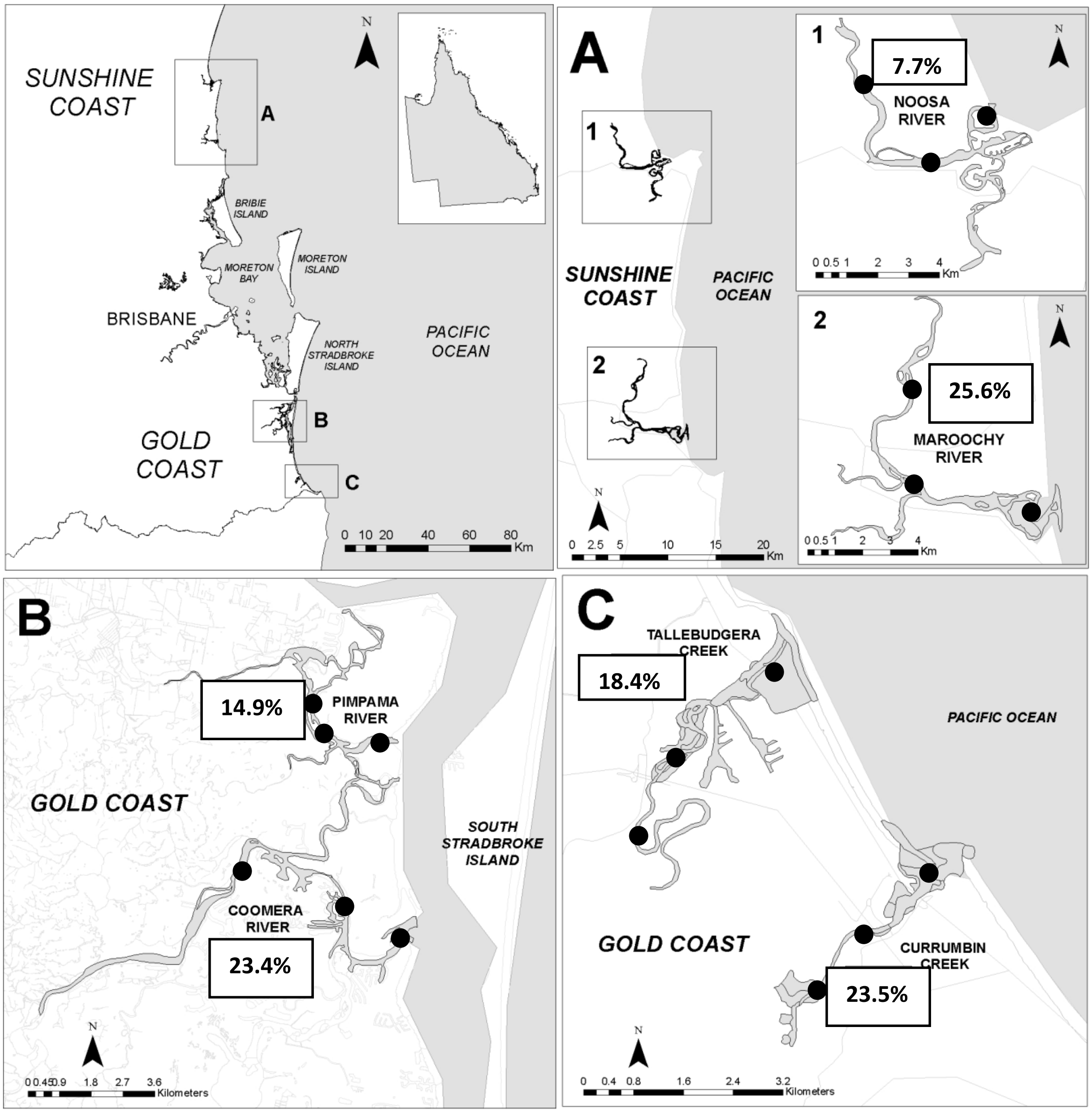

Figure 1. Sampling sites along estuaries of South-east Queensland with different urban values (%). (A) Noosa River (5.3%) and (2) Maroochy River (17.5%); (B) Pimpama River (11.0%) and Coomera River (19.1%); and (C) Tallebudgera Creek (13.9%) and Currumbin Creek (22.5%). The filled circles represent the three sampling sites in each estuary at the mouth, middle reach and upper tidal limit.

2.1.2 Other Australian estuaries

Due to the absence of reliable GIS data similar to the QLUMP and vegetation data used in the assessment of % urban in SEQ estuaries, the urban % of the catchments generating the literature $\delta^{15}N$ data were

2.2 Fish $\delta^{15}N$ data

2.2.1 SEQ estuaries

Between August 2012 and April 2013, five common fish species were collected from three sample sites along the tidal gradient of each SEQ estuary (mouth, middle reach and upper tidal limit) using a 25 m seine net. Up to six individuals of each target species, namely: sea mullet (*Mugil cephalus*), sand whiting (*Sillago ciliata*), yellow-fin bream (*Acanthopagrus australis*), silver biddy (*Gerres subfasciatus*) and estuary herring (*Herklotsichthys castelnaui*) were immediately euthanised in an ice-slurry and kept on ice before storage at -20°C for later analysis.

Tallebudgera and Currumbin Creeks were sampled at both the beginning (August 2012) and end (April 2013) of the study to investigate if the pattern of $\delta^{15}N$ changed significantly over the course of the study due to assimilation of new or different nutrient sources influenced by wet and dry seasons of the Southeast Queensland sub-tropical climate.

Fish were defrosted in the laboratory and dorsal white muscle (~5g), free from scale and skin, was removed from each fish and dried to a constant mass at 60°C. Using a mortar and pestle, each sample was homogenized into a fine powder prior to stable isotope analysis.

For stable isotope analysis, ~1 mg of homogenized fish tissue was accurately weighed into tin capsules and the $\delta^{15}N$ values were measured using a continuous-flow isotope-ratio mass spectrometer (GV Isoprime, Manchester UK; Inlet: Eurovector EA 3000, Milan, Italy) using atmospheric N as a standard.

The δ^{15}Nvalues, expressed as per mil (‰), were calculated using atmospheric N as standard:

$$\delta^{15}N\ (‰) = [(R_{sample} / R_{standard}) - 1]\ x1000$$

where R = ratio of the heavy to the light isotope, i.e.^{15}N/^{14}N.

2.2.2 Other Australian estuaries

Fish δ^{15}N data reported in the literature were collated from seven other Australian estuaries with urbanization ranging from 0.1% to 59%: Deluge Inlet, Queensland (0.1%; 18°25'S 146°12'E) (Abrantes and Sheaves, 2009), Mooloolah River, Queensland (13.1%; 26°41'S 153°8'E) (Schlacher et al., 2005), Nerang River, Queensland (21.3%; 27°59'S 153°25°E) (Connolly, 2003), Belongil Creek, New South Wales (15.8%; 28°38'S 53°36'E) (Hadwen et al., 2007); Towra Point, New South Wales (48.6%; 33°59'S 151°10'E) (Mazumder et al., 2011) and Swan River, Western Australia (59%; 32°3'S 115°44'E) (Linke et al., 2001). The δ^{15}N values of *M. cephalus* collected in the Logan (16.5%) and Albert Rivers (6.7%) (27°42'S 153°20'E) (Queensland), in a preliminary to this study, were also included. Fish Base (www.fishbase.org) was used to assign trophic level to all species represented in the literature and species were grouped into these trophic levels for analysis.

2.3 Statistical analysis

The relationship between urban % and δ^{15}N was analysed on individual species (*Mugil cephalus*: trophic level 2.1, *Sillago ciliata*: trophic level 3.2), as well as on *Herklotsichthys castelnaui* and *Gerres subfasciatus* combined, as these two species share the same trophic level (3.3) and feeding habits. *Acanthopagrus australis* was omitted at this stage of analysis due to low replicates across the sampled sites. Linear regressions were performed between δ^{15}N (mean ± standard error) and urban % of the catchment for each species (or species group) at each site within the estuary (mouth, middle and upper).

To test for differences in the relationships between urban % and fish $\delta^{15}N$ at the different estuarine locations (upper, middle and mouth), the slopes and elevations of regression lines generated for each location were compared using an analysis of covariance (ANCOVA). In cases where the slopes were parallel (ANCOVA: $p > 0.05$ for location × urban %,), a full factorial ANCOVA (location as the factor, $\delta^{15}N$ as the dependent variable, urban % as the covariate) was performed to test differences in the elevation of the slopes between locations. In cases where the ANCOVA was significant, least significant difference (LSD) post hoc analysis was used to identify which locations were significantly different, and the magnitude of the difference. No further analysis was done if the slopes were statistically different (ANCOVA: $p < 0.05$ for location × urban %).

In cases where there was no difference in the slopes and elevations of the regressions for the different locations (mouth, middle, upper), the data for each estuarine location were pooled to create a mean $\delta^{15}N$ (± standard error) for each species (or group) in each estuary. The relationships between urban % and the estuarine mean $\delta^{15}N$ were subsequently investigated for each species through a linear regression model. The slopes and elevations for the different trophic levels were then compared using a similar ANCOVA design to the one described above. To test for changes in $\delta^{15}N$ between wet and dry seasons, paired t-tests were run to compare the mean $\delta^{15}N$ of each species at each site (mouth, middle and upper) collected from Tallebudgera and Currumbin in 2012 to fish collected in 2013.

For testing a generic quantitative relationship between fish $\delta^{15}N$, Urban % and trophic level, data from the six estuaries in SEQ and the other seven Australian estuaries were analysed using a multiple regression model with Urban % and trophic level as predictor variables. The pooled data of the mean $\delta^{15}N$ of each species for each estuary were regressed against urban % in the first instance, using linear as well as polynomial (quadratic and cubic) models. Anderson and Cabana (2006) found that $\delta^{15}N$ values of consumers (including fish) from 82 rivers in Quebec, Canada, were best described by cubic or quadratic models. Secondly, trophic level (obtained from Fishbase, www.fishbase.org.) was added as a predictor in a multiple linear regression model. The $\delta^{15}N$ value is commonly used for estimating the trophic position of consumers such as fish, by assuming a fixed trophic enrichment factor across species and trophic levels, e.g. Post (2002), Minagawa and Wada (1984), Franca et al. (2011). The isotopically-derived values may not agree with conventional values derived from dietary (e.g. gut contents) analysis (Pasquaud et al., 2010). Apart from uncertainties about the value and the constancy of the trophic enrichment factor, this approach is problematic if the trophic position of the fish needs to be included together with urbanization as a determinant of their tissue $\delta^{15}N$, when trophic position is itself calculated from the observed $\delta^{15}N$ value. The Fishbase trophic level value is mostly obtained based on diet studies (http://www.fishbase.org/manual/english/FishbaseThe_ecology_Table.htm) independent of $\delta^{15}N$ measurements and would therefore eliminate this confoundment.

All statistics were performed in SPSS (v.21.) and SigmaPlot (v.11), and α was set at 0.05 for all tests.

3. Results

3.1 Southeast Queensland estuaries

The percentages of urban land in the catchment of the six estuaries were, in ascending order, 7.7 (Noosa), 14.9 (Pimpama), 18.4 (Tallebudgera), 23.4 (Coomera), 23.5 (Currumbin) and 25.6% (Maroochy).

3.1.1 Fish $\delta^{15}N$ values

The δ^{15}N values of all species were enriched with increasing urbanization, with the slopes and elevations of regression lines of the relationship not significantly different (ANCOVA, $p > 0.05$) between the upper, middle and lower parts of the estuary.

The δ^{15}N values of *Mugil cephalus* were enriched with increasing urbanization (%) at all three estuarine locations (mouth, middle and upper), with an increase of ~4.5‰ from the least urbanized Noosa River (~8‰) to more urbanized Currumbin Creek (~12.5‰) (Figure 2A). The regressions at both the middle and upper sites were statistically significant ($p<0.05$) (Middle: $R^2 = 0.65$, Upper: $R^2 = 0.88$). Although the trend was similar at the mouth, the relationship was not significant ($R^2 = 0.38$, $p > 0.05$), probably driven by the fact that *M. cephalus* was not captured at the mouth of the Noosa River or Tallebudgera Creek, thus reducing the sample size.

The δ^{15}N of *Sillago ciliata* also became enriched with increasing urbanization (%), from ~10 ‰ in Noosa River to ~13‰ at Currumbin Creek (Figure 2B). *S. ciliata* was commonly found at the mouth in all six estuaries, showing a significant positive linear relationship ($R^2 = 0.64$, $p \approx 0.05$). *S. ciliata* was found in the upper sites of all estuaries except Maroochy River. However, as with the mullet, sample size for Tallebudgera and Currumbin Creeks and Coomera River at the upper sites were too small to show a significant relationship ($R^2 = 0.65$, $p > 0.05$).

Gerres subfasciatus and *Herklotsichthys castelnaui* were grouped for analysis. The δ^{15}N became enriched (by ~3.5‰) between the least urban Noosa River (~10‰) to most urban Currumbin Creek (~13.5‰) following the same trend in the individual analysis of *M. cephalus* and *S. ciliata* (Fig. 2C). At the mouth, replicates of either species were only found in Tallebudgera and Currumbin Creeks and Pimpama and Coomera Rivers and there was a significant relationship ($R^2 = 0.93$, $p < 0.05$). Similarly, at the upper sites, samples were available from all estuaries apart from Maroochy River and showed a strong significant positive linear relationship between δ^{15}N and urban % ($R^2 = 0.98$, $p < 0.01$). *G. subfasciatus* was not found in the middle site of any of the estuaries. However, *H. castelnaui* was found at the middle of Pimpama and Coomera Rivers and Currumbin Creek (δ^{15}N: Pimpama River 7.1 ± 0.25‰; Coomera River 13.3 ± 0.58‰; Currumbin Creek 13.9 ± 0.78‰), suggesting that further replicates across the six estuaries would fit the emerging trend.

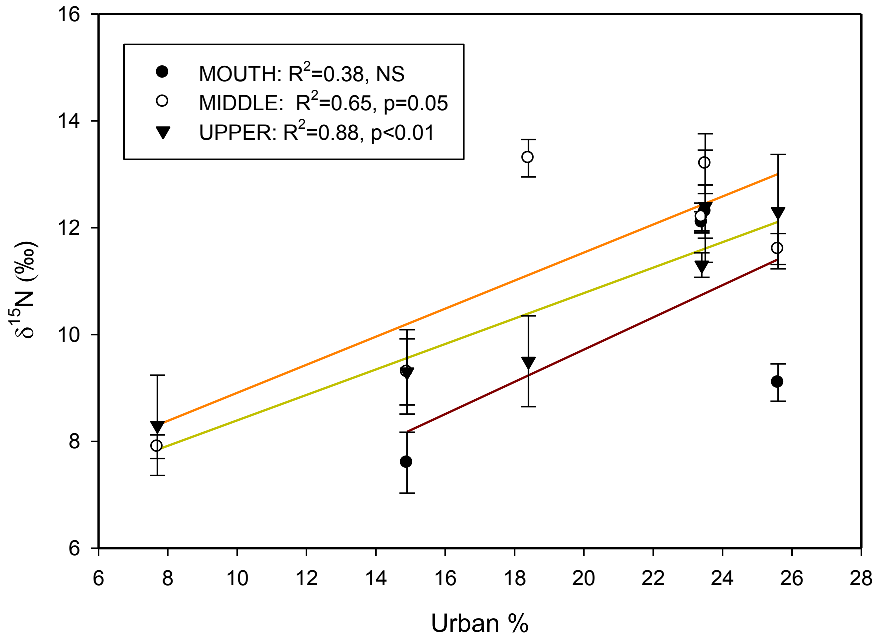

Figure 2A

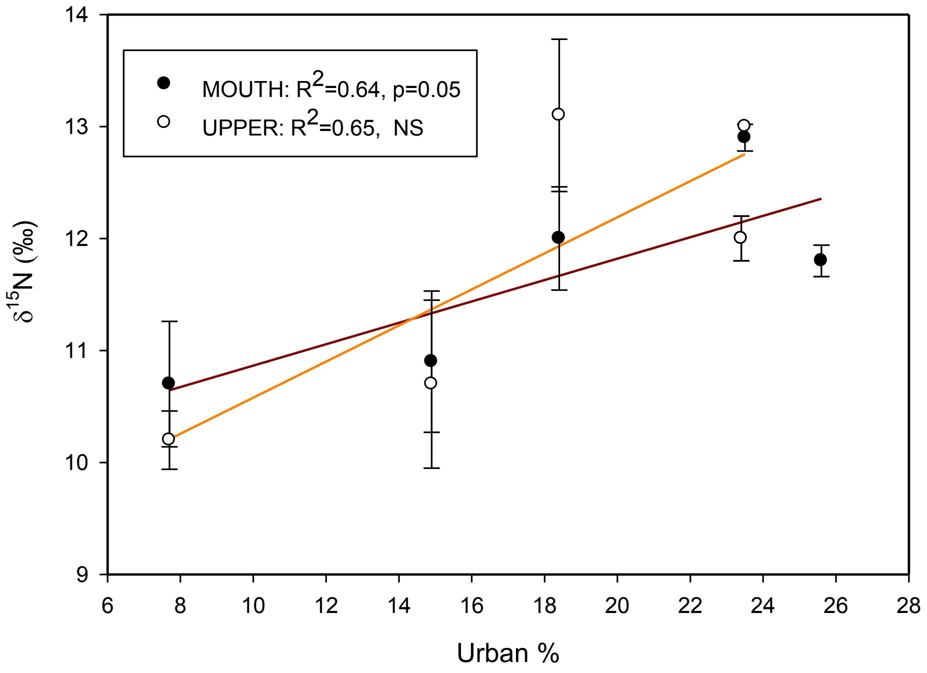

Figure 2B

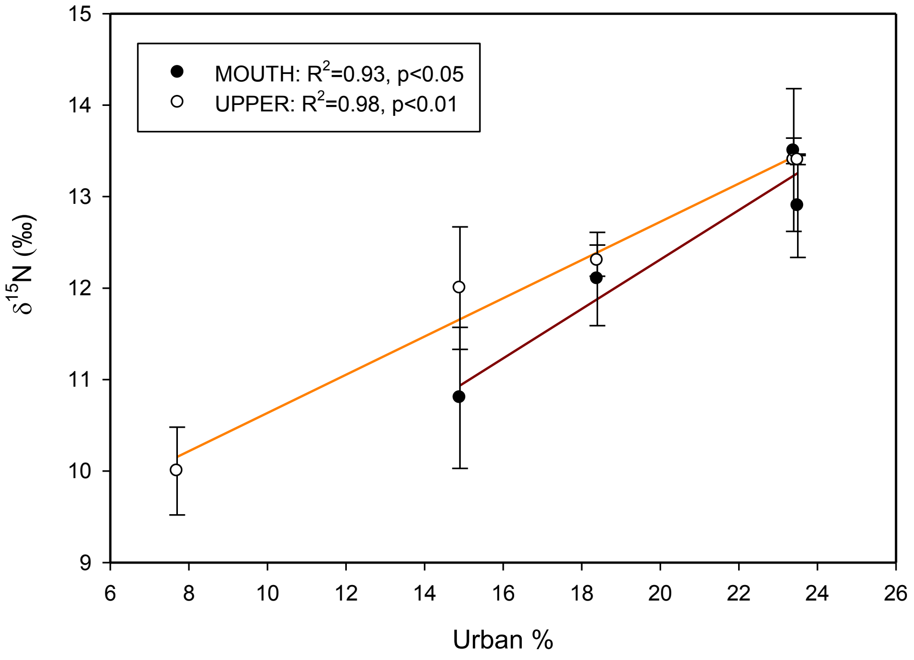

Figure 2C

Figure 2. Regressions of δ^{15}N (mean ± SE) against urban % for (A) *Mugil cephalus*; (B) *Sillago ciliata*; (C) *Gerres subfasciatus* and *Herklotsichthys castelnaui* collected from the mouth, middle and upper sites of six SEQ estuaries of differing urban development (see Fig. 1). The slopes and elevations between the sites were not significantly different (ANCOVA: $p > 0.05$) in all species.

The equality in slope and elevation of the different estuarine locations (mouth, middle and upper) for all species and groups allowed data for each estuary to be pooled. The mean estuary δ^{15}N values for *M. cephalus, S. ciliata* and *H. castelnaui* in each estuary were enriched increasing urban % with significant regressions ($R^2 \geq 0.68$, $p < 0.05$) (Figure 3). A 'universal' quantitative relationship between δ^{15}N and urban % can therefore be established for each species irrespective of where the samples were collected along the river course.

However, the slopes of these regressions were not parallel between trophic levels (ANCOVA, $p < 0.05$). At low urban %, δ^{15}N was generally enriched by ~2‰ from trophic levels 2 to 3 , while at higher levels of urban %, the δ^{15}N values of all trophic levels converged to a narrow range of ~12-13‰.

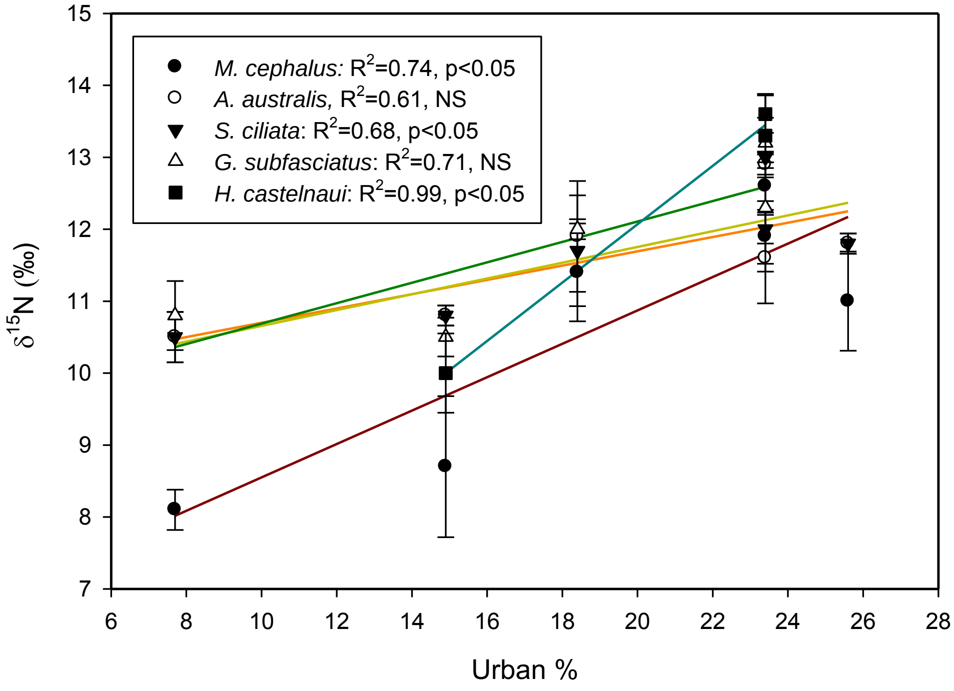

Figure 3. Mean estuary δ^{15}N (± SE) to urban % regressions for all species/trophic levels in the six SEQ estuaries. Results of the linear regressions indicate strong relationships for *Mugil cephalus* (R^2=0.87, p<0.01), *Sillago ciliata* (R^2=0.91, p<0.01) and *Gerres subfasciatus* + *Herklotsichthys castelnaui* (R^2=0.86, p=0.23). Note the narrowing difference between the δ^{15}N values of species at different trophic levels with increasing urban %.

3.1.2 Tallebudgera and Currumbin Creeks resample

Re-sampling of Tallebudgera and Currumbin Creeks showed no significant difference in mean δ^{15}N for *M. cephalus, S. ciliata, A. australis* or *H. castelnaui* caught in April 2013 and August 2012 (paired t-tests: p > 0.05). The pattern generated in the above analysis therefore probably applies to either season.

3.2 Other Australian estuaries

Regression of δ^{15}N against Urban % for the pooled data for Australian estuaries was significant ($\delta^{15}N = 9.39 + 0.105*Urban\%$; $R^2 = 0.717$, $p < 0.001$, Figure 4A). Quadratic or cubic polynomial models did not describe the data better than the linear model, as there is no significant change in the R^2 value (quadratic model R^2=0.717; cubic model R^2=0.722, both $p < 0.001$). A simple linear regression model is therefore sufficient to describe the relationship. Inclusion of trophic level (TL) further improves the variance in δ^{15}N explained by the significant positive linear relationship ($R^2 = 0.782$, $p < 0.001$; Figure 4B). Both % urbanization and TL of fish are significant predictors of δ^{15}N ($p < 0.001$), resulting in an overall regression relationship of $\delta^{15}N = 5.827 + 0.107*Urban\% + 1.197*TL$. δ^{15}N therefore increases monotonically with increases in either Urban % and trophic level.

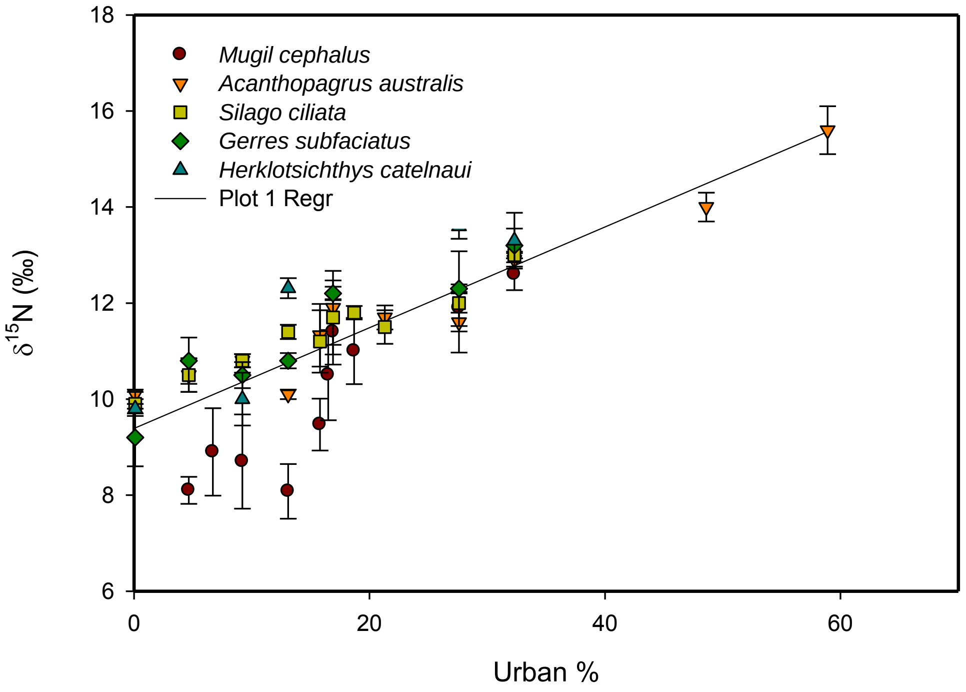

Figure 4A.

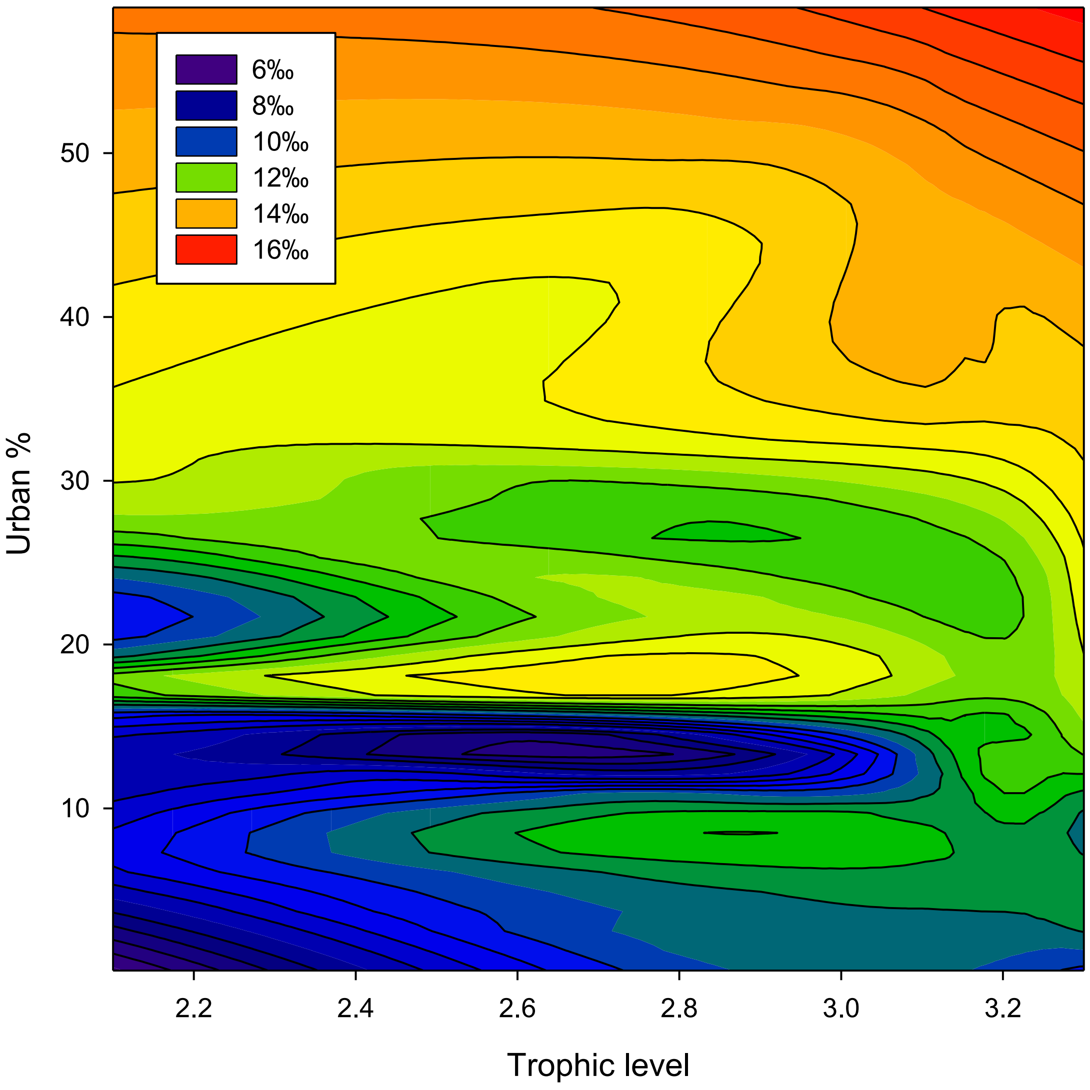

Figure 4B**.**

Figure 4. (A) Mean estuary δ^{15}N (± SE) to urban (%) regressions for all species/trophic levels of Australian estuaries of various levels of urban development showing δ^{15}N enrichment occurring with increasing urban %; (B) a three-dimensional contour plot based on the same data to illustrate the relationship. ● - *Mugil cephalus;* ■ - *Silago ciliata;* ◆ - *Gerres subfasciatus;* ▲- *Herklotsichthys castelnaui;* ▼- *Acanthopagrus australis.*

4. Discussion

4.1 Southeast Queensland estuaries

This study revealed a strong positive relationship between the amount of urbanization in a catchment and the $\delta^{15}N$ of the fish species within the estuary. Furthermore, this relationship was maintained over different trophic levels and at different locations along the estuarine tidal gradient, as well as when literature $\delta^{15}N$ values of fish from other Australian estuaries were combined with the data collected in this study. Fish stable isotope analyses in this study have therefore clearly illustrated that urban development in Australia is having an impact on the nutrient sources in the estuarine environment. The results clearly indicate that $\delta^{15}N$ is useful in revealing urbanization in an estuary, providing a quantitative relationship between enrichment and urbanization extent (more enriched = more urban). Our study suggests that there is a direct quantitative relationship between the degree of urbanization and the $\delta^{15}N$ values of fish, which has significant implications for the monitoring and management of nitrogen pollution in peri-urban estuaries.

Estuaries often receive a large amount of sewage output due to increased human activity within coastal zones and this excess nitrogen can be a contributing agent to estuarine ecological change (Schlacher et al., 2005). Estuarine animals exposed to effluent output have shown predictable shifts in $\delta^{15}N$, becoming more enriched with proximity to the output site because anthropogenic nitrogen sources, such as waste-water effluent, have more enriched $\delta^{15}N$ than natural sources (Piola et al., 2006; Pitt et al., 2009; Schlacher et al., 2005; Schlacher et al., 2007). Estuaries with numerous nitrogen sources (e.g. industrial, sewage treatment, agriculture and stormwater run-off) show further $\delta^{15}N$ enrichment due to trophic discrimination occurring during numerous nitrogen transformations, which take place at higher rates in urban estuaries compared to natural estuarine sites (Schlacher et al., 2005). It could therefore be the combined effects of multiple urban nitrogen sources, rather than just sewage, that have the strongest effect on nitrogen enrichment and the δ^{15}N value of consumers in peri-urban estuaries. This was demonstrated in this study, as fish from low % urban estuaries with sewage treatment plants (STPs), such as Maroochy and Tallebudgera, had more depleted δ^{15}N than some higher % urban estuaries with no STPs, such as Currumbin and Coomera. In a recent study covering 84 sites in New Zealand, Clapcott et al. (2010) found that δ^{15}N of stream primary producers was strongly affected, in descending order, by nitrogen concentration (log-transformed, log N), % removal of native vegetation and % impervious cover in the catchment. Relationship between these factors and δ^{15}N of primary producers were, however, mostly curvilinear. For example, log N increases δ^{15}N monotonically until the threshold of ~3.2 mg L^{-1} (log N = 0.5) but plateaued thereafter. While urbanization (as indicated by % impervious cover) apparently has a weaker influence than N load and vegetation changes, their study involved areas generally with little urbanization, with a mean % of impervious cover in catchment at 5% (Clapcott et al., 2010).

The trend of fish δ^{15}N values was also consistent throughout the tidal range of estuaries, with positive linear relationships between urban % and δ^{15}N at each sampling location (mouth, middle and tidal limit). This suggests that the movement of fish between these sites, and the subsequent distribution of nutrients, are constant factors within the estuary that reflect a time-integrated average of conditions regardless of any one particular nutrient input site. These results are therefore in contrast to some previous δ^{15}N fish studies that argue sewage treatment plants in particular, are main drivers of δ^{15}N enrichment in estuarine systems and therefore could be used to indicate the extent of urbanization (Piola et al., 2006; Pitt et al., 2009; Schlacher et al., 2005). We therefore suggest that it is in fact the combined effects of multiple urban nitrogen sources having the strongest effect on δ^{15}N of estuarine fish. Anderson & Cabana (2006) concluded from a study involving 82 river sites in eastern Canada that while $\delta^{15}N$ was a good indicator of anthropogenic N discharge into urban estuaries, this metric is incapable of resolving the relative importance of individual N sources, e.g. agricultural runoff and fertilizer use. Ogawa et al. (2001) demonstrated a significant positive correlation between $\delta^{15}N$ of a gobiid fish and human population growth in the Lake Biwa area, Japan, during the period 1910 and 1994 and attributed the increase in $\delta^{15}N$ to increased N load and/or enhanced denitrification associated with urban growth.

This relationship between urbanization and multiple anthropogenic nitrogen inputs to receiving estuaries was illustrated by the highly significant linear relationships between the degree of urbanization (%) and $\delta^{15}N$ values for all fish species. This strong relationship suggests that increase in $\delta^{15}N$ is directly proportional to the amount of urbanization in the catchment, which further establishes the usefulness of this parameter as a quantitative indicator of estuarine impact. Similarly, highly significant linear relationships between $\delta^{15}N$ and increasing urban % across different trophic levels demonstrate that the impact of urbanization on fish $\delta^{15}N$ is consistent throughout the food web. Other factors such as fish size are known to potentially influence $\delta^{15}N$ values (Reum and Marshall, 2013). Variation about the regression line is expected to increase if a wider range of body sizes is used for analysis. Significant ontogenetic shifts in feeding habit and thus, trophic position, may drive changes in $\delta^{15}N$ with body size. However, recent data on potential effect of species and size of fish on their trophic position (as indicated by the $\delta^{15}N$ value) in 23 streams in eastern Canada suggest that neither fish size or species identity influenced the response of their trophic position towards anthropogenic impact (Anderson and Cabana, 2009). Similar lack of effect of fish size on $\delta^{15}N$ has been reported for individuals in captivity (Vinagre et al., 2011) as well as species for which significant seasonal and inter-site variations have been reported (Olin et al., 2012).

Uniformity in response of $\delta^{15}N$ among animal taxa exposed to environmental change also seems to be common. An analysis of the enrichment pattern of $\delta^{15}N$ to sewage pollution on the Adelaide coast, south Australia, suggests that enrichment was uniform not only among related species, but organisms from different phyla (Connolly et al., 2013). Similar consistent variations in $\delta^{15}N$ among different taxa of animals in response to anthropogenic N enrichment have also been reported (Nixon et al., 2007). Our study collected fish individuals with a relatively narrow range of sizes, further minimizing the potential influence of this factor on the reported relationship.

Although we observed a strong relationship between urban % and fish $\delta^{15}N$ values across a range of trophic levels, the magnitude of the difference in $\delta^{15}N$ between trophic levels was lower than the mean discrimination value of 3-4‰ generally reported in the literature (Fry, 1991; Post, 2002). In addition, the differences in $\delta^{15}N$ between trophic levels were not strictly consistent over the urban % gradient. For example, trophic shift in Currumbin Creek between trophic level 2.1 (*M. cephalus*) and trophic level 3.3 (*G. subfasciatus and H. castelnaui*) was 0.7‰, while in the least urbanized estuary (Noosa) trophic shift was ~2.7‰. A possible explanation for this is that increased urbanization to a system also impacts the rate at which nitrogen is assimilated by consumers including estuary fish regardless of trophic level, with lower trophic level fish in an urban estuary showing a quicker metabolic uptake of nitrogen sources than those found in less urban sites. Alternatively, lower trophic level species such as mullet may feed at slightly higher trophic levels at sites with significant N enrichment. Indeed, any biotic or abiotic change to an estuary has the capacity to affect niche production or elimination that could elicit a wide range of responses in fish including species distribution, feeding and growth (Whitfield and Elliot, 2002). However, with no empirical evidence in the literature to suggest fish change their trophic position in response to urbanisation, this response would only be speculative. In addition, a link between urbanisation and a change in fish trophic level is not supported by recent data, e.g. Anderson and Cabana (2009), who found that the slope of the δ^{15}N-fish size relationship was positively influenced by chlorophyll a concentration (as an indicator of productivity and N input), but fish trophic position was not significantly affected. Where significant anthropogenic N enrichment occurs, inter-site variability in fish trophic position (e.g. Franca et al. (2011) may simply be a result of N pollution rather than shifts in the feeding ecology of the species.

4.2 Australia-wide analysis

In the second part of this study, we demonstrated that the relationships between % urbanization and fish δ^{15}N across multiple trophic levels were maintained when literature fish δ^{15}N data from other estuaries were combined with our data from SEQ. As was found in the initial six estuaries, the literature fish δ^{15}N accurately reflected the impacts and extent of urbanization to each system with enrichment of δ^{15}N occurring with increasing urban %. In fact, the addition of estuaries in the literature extended the range in % urban from ~0% in the least urbanized Deluge Inlet (National Land and Water River Audit (NLWRA) classified 'pristine') to ~72% in the highly urbanized Swan River (NLWRA classified 'extensively modified'), with a difference of 6.5‰ δ^{15}N in the fish from these two sites. The results of the multiple regressions including trophic level further validated this relationship between fish δ^{15}N and increasing urban %, with highly significant positive linear relationships occurring for all species in all estuaries. Overall, this study suggests that a universal relationship between fish δ^{15}N and % urbanization exists for any trophic level when the extent of urbanization to a system was known. This supports use of fish as bio-indicators, adding an extra ecological dimension to traditional water quality monitoring techniques to assess the condition of an estuary with meaningful urban impact integration.

With the incorporation of trophic level data of the species involved, our regression model suggests that the $\delta^{15}N$ values of estuarine fishes can be predicted irrespective of feeding habit, occurrence within the estuary, and location, for estuaries in Australia. The general applicability of this model, however, needs to be tested further using data sets with wider geographic, taxonomic and ecological coverage. This empirical relationship, if generic, would support the use of $\delta^{15}N$ values of fish as a universal quantitative indicator of urbanization.

Estimation of urban % using Google Earth as described here also provides an easily accessible, universal approach to estimating urbanization in catchments world-wide. This database has been used for similar large-scale spatial analyses (Huang et al., 2009; Monkkonen, 2008; Seto et al., 2011). The Google Earth approach was validated through the strong correlation with the more accurate, but less accessible, QLUMP data, and therefore supports the universal use of Google Earth for land use analysis. This will allow estuarine and catchment managers to easily assess and predict the impact of urbanization on estuarine trophodynamics, and allows all future studies on the $\delta^{15}N$ of estuarine fish to be assessed against the model presented here. This is an important consideration in estuarine monitoring and management given the rapidly increasing global population and expansion into coastal zones.

5. Conclusion

The consistent enrichment in fish $\delta^{15}N$ with increasing urban % and the development of a quantitative model that predicts $\delta^{15}N$ with known urban % and trophic level adds a new ecological tool to the evaluation of estuarine and coastal zone conditions in the presence of growing urban development. Importantly, this model is based on multi-species and multi-trophic level analyses rather than commonly used single species bio-indicators, creating a more generic method of monitoring using δ^{15}N as a quantitative indicator of urban impact in estuaries.

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