

# **Biotic responses to urbanisation in mangrove dominated estuaries**

Ruth Anne Young, BSc (Hons)

Griffith School of Environment,  
Faculty of Science, Environment, Engineering & Technology,  
Griffith University

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## Abstract

Increasing urbanisation of coastal areas is placing unprecedented pressure on estuarine environments. Impacts associated with urbanisation can alter the health of estuarine ecosystems, reducing their capacity to provide valuable ecosystem services. In order to protect these environments, relationships between estuarine health and urbanisation should be understood and applied to sustainable urban planning in coastal areas. Responses measured in estuarine biota offer much potential for providing pragmatic and sensitive means for the detection of urban impacts in estuaries.

I assessed the responses of estuarine biota to increasing levels of urbanisation in the rapidly developing coastal region of southeast Queensland, Australia. I measured a suite of biotic indicators, namely: the  $\delta^{15}\text{N}$  of mangroves and crabs as a tracer for urban N sources, sediment chlorophyll *a* concentration (Chl *a*) as an estimate of the biomass of microphytobenthos (MPB), and a condition index in crabs, measured as the hepatosomatic index (HI). Urbanisation was estimated as the percentage of catchment covered by impervious surfaces (impervious cover, IC). Increases in impervious surfaces such as roads, footpaths and car parks are closely linked to urbanisation. These surfaces concentrate and convey pollutants generated from urban activities into coastal waterways and estuaries, thus making IC a useful indicator of urban impact.

In a case study of Lake Coombabah, an estuarine lake situated in a rapidly urbanising catchment, I identified three influx sites where urban runoff was likely to enter the lake. In order to assess the impacts of urban pollution on biota around these sites, I tested the model that mangroves (*Avicennia marina*) and crabs (*Australoplax tridentata*) located near (< 10 m) the influx sites would display a greater assimilation of urban  $\delta^{15}\text{N}$  than mangroves and crabs located farther away (250 m) and that MPB biomass and crab condition would differ between distances near and far from the influx sites. Enriched  $\delta^{15}\text{N}$  values in mangroves ( $4.1 \pm 0.2\text{‰}$ ) and crabs ( $6.7 \pm 0.2\text{‰}$ ) at one of the influx sites, which was located near a sewage treatment plant (STP), indicated that N from treated sewage was entering the lake's food web. Enrichment of mangrove and crab  $\delta^{15}\text{N}$  values at the other two influx sites following rainfall also indicated some contribution of N from urban runoff. This enrichment of  $\delta^{15}\text{N}$  occurred in biota near and far from the influx sites, indicating that urban runoff

penetrates at least 250 m into the lake. A faster recovery to pre-rainfall  $\delta^{15}\text{N}$  values in mangroves and crabs located 250 m from these influx sites indicated a longer residence time of urban N closer to these sites. The condition of crabs at the influx site near the STP fluctuated more through time than it did 250 m away, potentially because exposure to sewage N and other pollutants is more acute at this influx site. This study indicated that urban runoff and treated effluent from the adjacent STP is starting to have an effect on the lake's nutrient sources and on the condition of consumers, at least at a localised scale around the urban influx points.

In order to assess whether urban growth around the region was having an increasing impact on estuarine biota, I tested whether the indicators showed a relationship with increasing levels of urbanisation (IC) surrounding multiple estuarine systems in both lake ( $n = 5$ ) and creek ( $n = 8$ ) settings. In the estuarine lakes I measured these responses over a one-year period, three times during the wet and dry seasons. Both studies indicated that STPs and septic tank systems convey urban sources of N to estuaries. A slight enrichment of  $\delta^{15}\text{N}$  in biota in urbanised creeks that were not exposed to sewage effluent indicated that urban runoff also contributes nutrients to these estuaries. The assimilation of these urban sources of N, however, had no effect on the MPB biomass and condition of crabs in the lakes or the creeks. Instead, these indicators displayed negative relationships with impervious cover. The responses of MPB biomass and crab condition were strongly correlated, indicating a trophic cascade of urban impacts between these biota.

The combination of MPB biomass and crab condition into an index of biotic condition (BCI) also showed a negative relationship with greater catchment urbanisation. In lakes, this response was dependent on the relative size of the catchment and the lake as well as IC ( $r^2 = 0.79$ ), but in creeks most of the variability in biotic condition was explained by the extent of urbanisation (IC) alone ( $r^2 = 0.61$ ). The condition of biota in the lakes showed a rapid power shaped decline when IC exceeded 4%, whereas in the creeks, biotic condition showed a more linear decline with greater IC. These different responses may have been due to hydrological differences between the lakes and the creeks and highlight the importance of considering system-specific attributes when applying IC as a predictor of urban impact. The absence of any significant response in the BCI to the assimilation of sewage N, together with the negative response to greater IC, indicates that pollutants

conveyed in urban runoff are having a greater impact on the condition of mangrove biota than exposure to nutrients from treated sewage.

Urbanisation leads to increases in sediment and nutrient loads in estuaries. The potential counteractive interactions between these pollutants were explored in a mesocosm experiment in which sediment and N were added at levels typical of urban runoff scenarios. MPB biomass showed no response to N additions ( $3.2 \text{ mg L}^{-1}$ ) but decreased significantly in response to sediment additions ( $3 \text{ g L}^{-1}$ ), supporting the model that, under the experimental conditions, sediment delivered in urban runoff has a greater effect on MPB production than nutrients. Together with other studies in turbid estuaries these results indicate that primary production may be nutrient replete and therefore limited more by light in these environments. Crab condition showed no response to sediment or N additions. Crabs exposed to sediment additions appeared to obtain their nutrition from MPB detritus but, in the longer term, as the crabs graze down the diminishing stock of living and dead MPB, their condition may also eventually decrease.

The biotic indicators measured in this thesis demonstrate that urbanisation around southeast Queensland is affecting the biota of mangrove-dominated estuaries. Urban sewage has altered the nutrient supply to the food webs in these environments. However, sediment loads, appear to have the greater effect on benthic primary production in these systems. The MPB and crabs measured throughout this study contribute importantly to the structure and function of mangrove ecosystems. Impacts to their biomass and condition are likely to lead to implications for the health of these systems. In order to maintain the essential services provided by these estuaries, continuing urban growth must be managed in a manner which conserves their health. Long-term monitoring of estuarine health will assist managers in gauging the effects of urbanisation, as well as the success of pollution management practices. As our understanding of ecology evolves, novel approaches to monitoring ecosystem health are emerging. The biotic indicators measured in this thesis offer a cost effective snap shot method of detecting subtle estuarine responses to increasing urbanisation.



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## **Declaration**

The material presented in this thesis has not been previously submitted for a degree or diploma in any university, and to the best of my knowledge contains no material previously published or written by another person except where due acknowledgement is made in the thesis itself.

Ruth Young



# Chapter 1. Introduction

## 1.1 Overview of the thesis

Estuaries provide valuable ecosystem services to humankind (Costanza et al. 1997). These services, however, are under threat due to human alteration of coastal landscapes which can degrade the health of estuarine ecosystems. Globally, in the last 40 years the human population has doubled and approximately 60% of this population resides within 100 km of the world's coasts (Vitousek et al. 1997). Land 'reclamation' associated with urbanisation may involve the direct destruction of coastal ecosystems such as mangrove forests (Alongi 2002). Urbanisation, however, also leads to drastic landscape changes throughout the catchments of receiving estuaries. These include the clearance of forested areas, with subsequent increases in the ratio of impervious to pervious land surfaces. These landscape changes alter catchment hydrology, increasing the runoff of stormwater and urban pollutants into coastal waters, often resulting in degraded estuarine health (CWP 2003, Halpern et al. 2008).

Urban pollution degrades the ecosystem health of estuaries by altering their nutrient sources, system organisation (e.g., species composition), vigour (e.g., primary productivity) and, by reducing their resilience, i.e., capacity to counteract disturbance (Rapport 1998b, Holland et al. 2004). In order to manage coastal urbanisation in ways which prevent further degradation of estuaries, we need to monitor the health of estuarine environments as urbanisation continues and instigate appropriate action. Previous monitoring has involved both individual and integrated measures of abiotic and ecological variables (e.g., Sanger et al. 1999a, b, Holland et al. 2004, Bilkovic & Roggero 2008). To date, many ecological responses have focused on measures such as community composition, which rely on reductions in the abundance and local extinction of sensitive taxa. Such measures, however, only indicate an impact when an ecosystem has already changed to a degraded state i.e., reduced system organisation and resilience. Restoration of already degraded ecosystems is difficult, expensive and seldom possible, so there is a need for indicators that can detect signs of degradation early, preferably before the structure (e.g., biodiversity) or the state (e.g., trophic status) of an ecosystem has changed. Responses measured in estuarine biota before their abundance has declined, or before an impact has made them locally extinct, are likely to provide a more sensitive indication of urban impact, enabling action before an ecosystem has changed to a degraded state (Schlacher et al. 2007).



The coastline of southeast Queensland, Australia, has diverse estuarine environments, ranging from coastal embayments with extensive seagrass beds to small mangrove-lined tidal creeks. The ecological significance of these environments is recognized in that much of the region's coastline is included within the Moreton Bay Marine Park. Southeast Queensland is, however, one of the fastest growing regions in Australia, and rapid urbanisation of its coasts is already impacting the water quality of estuaries in the area (EPA 2007). While the water quality of estuaries in the region is monitored by the Queensland Environmental Protection Agency (EPA) and local catchment care groups, there has been no specific investigation of biotic or ecosystem level responses to urbanisation.

This thesis assesses the responses of biota to increasing urbanisation in mangrove dominated estuaries around southeast Queensland. A suite of indicators that are likely to provide an early warning signal of ecosystem degradation were measured in mangrove biota to detect impacts in response to increased urbanisation. These indicators included an isotopic tracer to provide an indication of altered nutrient sources, the biomass of benthic microalgae (as a proxy for benthic primary production), and a condition index in estuarine consumers. The responses of these indicators were measured at multiple spatial and temporal scales and in different types of estuarine systems, typical of subtropical southeast Queensland.

## **1.2 Estuarine environments offer valuable ecosystem assets**

Estuaries form an interface between land and sea, occurring where terrestrial freshwaters converge with marine waters. The diverse abiotic conditions formed by the mixing of fresh and saline waters has led to the evolution of ecosystems comprising biota adapted to a life of extremes in salinity, oxygen, temperature, and light fluctuations. These ecosystems are typically made up of mangrove forests, salt marshes, seagrass beds, mudflats, and sandflats.

Estuarine ecosystems provide services that are valuable to human welfare (Costanza et al. 1997, Rapport et al. 1998, Valiela et al. 2001). They include the storage and redistribution of floodwaters, protection from storm surges and tsunamis, the breakdown and transformation of pollutants, the capture of solar energy and nutrients to provide food and energy, and the provision of varied habitats to many invertebrate, fish, bird, reptile and mammal species (Chaves & Bouchereau 2000, Valiela et al. 2001, Valiela & Cole 2002, Dahdouh-Guebas et al. 2005, Krauss et al. 2008, Nagelkerken et al. 2008). In a report by Costanza et al. (1997), the economic value of the services provided by the world's estuaries was estimated at US\$4.11 trillion per year. Growing human inhabitation of the world's

coastlines has altered and degraded many estuarine ecosystems, including the transformation and loss of ~ 50% of the world's mangroves (Vitousek et al. 1997, Valiela et al. 2001). As these ecosystems become degraded, their capacity to provide ecosystem services is reduced (Rapport et al. 1998).

### **1.2.1 Mangrove forests**

Mangrove forests grow in the intertidal zone of sheltered estuaries, forming a dense fringe of salt-tolerant trees at the land-sea interface (Valiela et al. 2001). Mangroves are widely distributed throughout tropical and subtropical regions and generally grow between latitudes of 25°N and 25°S (Valiela et al. 2001, Connolly & Lee 2007). Due to the typically poorly aerated substrate of mangrove forests, mangroves have developed a number of adaptations for surviving in hypoxic conditions, including the production of aerial roots (Lee 2008). The complex landscape formed by these roots and soft sediments provides habitat to diverse communities, including many commercial fish and invertebrate species (Nagelkerken et al. 2008). The intertidal flats of mangroves are covered by single-celled epipelagic diatoms and cyanobacteria (microphytobenthos, MPB), while the aerial roots of mangroves support epibionts including algae, sponges, corals and bivalves (MacIntyre et al. 1996, Nagelkerken et al. 2008). The structure of the roots also provides refuge for many mobile fish and invertebrate species (Chaves & Bouchereau 2000, Nagelkerken et al. 2008). Insects and birds inhabit the mangrove canopy, while macrofauna such as gastropods, oligochaetes, and brachyuran crabs dominate the soft sediments (Lee 2008, Nagelkerken et al. 2008).

While mangrove forests are highly productive, they may not be the major source of food for the animals found within them. Initially, the high productivity of mangroves led to the formulation of the “mangrove out-welling” model, which states that mangrove leaf litter is the predominant food source in estuaries and in near-shore waters due to tidal out-welling of mangrove detritus (Odum & Heald 1972).

Although mangrove leaves are rich in carbon, they offer little nitrogen (N) content and also contain grazing deterrents such as polyphenolics and tannins, making them a less appealing food source (Neilson & Richards 1989, Skov & Hartnoll 2002, Thongtham & Kristensen 2005). Furthermore, a recent review of dietary studies using stable isotope tracers revealed a lack of unambiguous evidence supporting the use of mangrove detritus as a major energy source in estuaries and near-shore environments (Lee 2005, Bouillon et al. 2008). Recent evidence suggests that estuarine consumers obtain their energy from a variety of sources. These include benthic microalgae, mangrove leaf and propagule litter, epiphytic

algae, other fauna, suspended matter, and imported material (France 1998, Lee et al. 2001, Bouillon et al. 2004, Proffitt & Devlin 2005, Nordhaus & Wolff 2007). While mangrove leaves are likely to be an important carbon source in terms of availability, they may not be the most nutritious or assimilated food source in estuaries. It should be noted, however, that mangroves are important in providing and maintaining an environment for alternative carbon sources. For instance, the aerial roots of mangroves provide habitat for epiphytic algae and also trap sediments, leading to the formation of mudflats that support MPB production (Tomlinson 1986).

### **1.2.2 Brachyuran crabs: the consumers of the forest floor**

Brachyuran crabs are ubiquitous consumers of the mangrove epibenthos (Snelling 1959, Tan & Ng 1994, Lee 1995, Hartnoll et al. 2002, Skov et al. 2002, Lee 2008). Due to their abundance, crabs have a significant influence on the structure and function of mangrove ecosystems and are often described as ecosystem engineers (Jones et al. 1994, Kristensen 2008). By increasing the surface area of sediment exposed to air and oxygen rich water, the burrowing activities of crabs enhance important biogeochemical processes that are sensitive to redox conditions (Fenchel 1996, Cannicci et al. 2008, Lee 2008). These changes to sediment chemistry can affect mangrove growth and production (Smith 1987, Kristensen & Alongi 2006). Crabs may also influence forest structure by selectively grazing on mangrove propagules (Smith 1987, Cannicci et al. 2008). The feeding and burrowing activities of crabs can also influence benthic primary production due to the removal and burial of MPB (Taylor & Allanson 1993, Armitage & Fong 2006).

The feeding activities of grapsid and ocypodid species also enhance the recycling of mangrove detritus by transforming the leaves into finely fragmented faecal material. This increases the surface area to volume ratio of the leaves and facilitates their microbial breakdown, accelerating the decomposition and recycling of mangrove material within the system (Lee 1997, Werry & Lee 2005, Nordhaus & Wolff 2007). Due to their consumption by mobile predators such as fish and shorebirds, the crabs are also likely to provide an important trophic link between primary producers and predators within estuaries and in adjacent near-shore environments (Piersma 1986, Sheaves & Moloney 2000, Zharikov & Skilleter 2004, Lugendo et al. 2006).

A more novel idea is that the reproductive outputs of mangrove crabs transfer energy from primary producers in mangrove environments to predators in estuarine and nearshore waters (Lee 2008). Small grapsid crabs produce 10,000 – 25,000 eggs (~0.05 g AFDW) per

brood and may produce at least 2 – 3 broods per year (Lee & Kwok 2002, Colpo & Negreiros-Fransozo 2003). Due to the abundance of these crabs, this reproductive output is likely to provide a significant export link from plant matter in mangrove ecosystems to consumers in near-shore systems (Lee 2008). While little is known about this link, a similar trophic linkage has been reported within estuaries between crab zoea and fish (*Ambassis jacksoniensis*) visiting inundated saltmarshes in southeast Queensland (Hollingsworth & Connolly 2006).

The mangrove environments of southeast Queensland support a diverse array of brachyurans. These include large portunid species such as the mud crab, *Scylla serrata*, which is thought to contribute substantially to the cycling of energy within higher trophic levels both in its role as a consumer of fish, crustaceans and carrion and as a source of prey to larger predators such as bull sharks (*Carcharhinus leucas*) (Perrine 1978). Many ocypodid crabs also inhabit the banks of mangrove lined estuaries around the region, including several species of fiddler crab (*Uca* spp.), the semaphore crab (*Heloecius cordiformis*) and the tuxedo crab (*Australoplax tridentata*) while species such as sentinel crabs (*Macrophthalmus setosus*) live in the soft intertidal mudflats. Most of these ocypodids are thought to feed on MPB (Rodelli et al. 1984, France 1998, Bouillon et al. 2002a). A variety of grapsid crabs also live in these environments, such as the red-fingered marsh crab (*Parasesarma erythrodictyla*), maroon mangrove crab (*Perisesarma messa*) and the scarlet three-spined crab (*Neosarmatium trispinosum*). Studies on similar species indicate that grapsids consume substantial amounts of mangrove leaf material but may actually derive more energy from MPB and other small invertebrates (Davie 1998, Dahdouh-Guebas et al. 1999, Bouillon et al. 2002a).

*Australoplax tridentata* (family Ocypodidae) and *Parasesarma erythrodictyla* (family Grapsidae) appear to be the most ubiquitous brachyuran species in mangrove habitats around southeast Queensland (Snelling 1959). A study by Guest et al. (2004) indicated that in mangrove forests, both species obtain their energy from MPB or a combination of MPB, mangrove material and detritus from adjacent saltmarsh. *P. erythrodictyla* are thought to play an important role in the cycling of mangrove material but only assimilate 12% of the leaf material ingested, adding support to the idea that mangrove leaves are not the most assimilated food source in estuaries (Camilleri 1989). Nonetheless, the transformation of leaves into finely fragmented faecal material by *P. erythrodictyla* is likely to enhance their microbial breakdown and decomposition (Werry & Lee 2005). Therefore, whilst only deriving a small percentage of nutrition from mangrove leaves for itself, *P. erythrodictyla*

may contribute significantly to the microbial cycling of mangrove leaf material. While few studies have specifically examined the diet of *A. tridentata*, it is thought that this species derives most of its nutrition from MPB, similar to other ocypodid species (Rodelli et al. 1984, France 1998). Signs of extensive bioturbation activity are evident in the intertidal zone for both crabs (pers. obs.) and previous studies on similar species (e.g. Sheaves & Moloney 2000, Lugendo et al. 2006) indicate that the crabs are likely to form an important trophic link between primary producers and consumers. Due to their abundance, both species are likely to contribute significantly to the structure and function of mangrove ecosystems around southeast Queensland.

### **1.3 Threats to estuarine environments**

Human activity has altered between one third and one half of the earth's land surface (Vitousek et al. 1997). The concentration of human activities in coastal areas has led to some degree of degradation to most of the earth's coastal ecosystems. Approximately 50% of the world's mangrove forests have been cleared or transformed by human activity (Vitousek et al. 1997, Halpern et al. 2008).

Landscape changes within the estuarine catchment also affect the runoff of pollutants and nutrients into coastal waters (Halpern et al. 2008). Increased N inputs from agriculture and urban sewage systems have led to eutrophication and algal blooms in estuaries and bays around the world (Cloern 2001, Beman et al. 2005). Increased flows of other agricultural, industrial and urban pollutants such as pesticides, metals, petrochemicals and sediments, inhibit primary production and also reduce the survival of many biota (MacIntyre et al. 1996, Leung & Furness 2001, Moreno-Garrido et al. 2003, Elumalai et al. 2005, Airoidi & Hawkins 2007, Mangialajo et al. 2008). These impacts affect the health of estuarine ecosystems, increasing their risk of switching to degraded states (Rapport et al. 1998, Scheffer et al. 2001).

### **1.4 Coastal urbanisation: altered landscapes**

Urbanisation radically changes the landscape of coastal catchments. These changes include forest clearance and the replacement of pervious surfaces with impervious surfaces such as roads, pavements, buildings and car parks. Impervious surfaces reduce the infiltration of rain water into naturally pervious soils and therefore, increase the channelling of rainwater and urban pollutants into receiving waterways (Schueler 1987, Arnold & Gibbons 1996, CWP 2003). This urban runoff contains a cocktail of heavy metals, polycyclic aromatic hydrocarbons (PAH), mineral oil hydrocarbons (MOH), sediments, nutrients, herbicides and

increased rainwater flows (Drapper et al. 2000, CWP 2003, Blanchoud et al. 2004, Gobel et al. 2007). The increase of urban runoff can also lead to greater salinity fluctuations in estuaries (Holland et al. 2004). The percentage of catchment covered by impervious surfaces (Impervious Cover, IC) is positively related with housing density, as well as urban pollutant loads in receiving waterways (Arnold & Gibbons 1996, Walsh et al. 2001, CWP 2003), Figure 1.1. This makes it a convenient estimate of urbanisation and its potential impacts in estuaries.

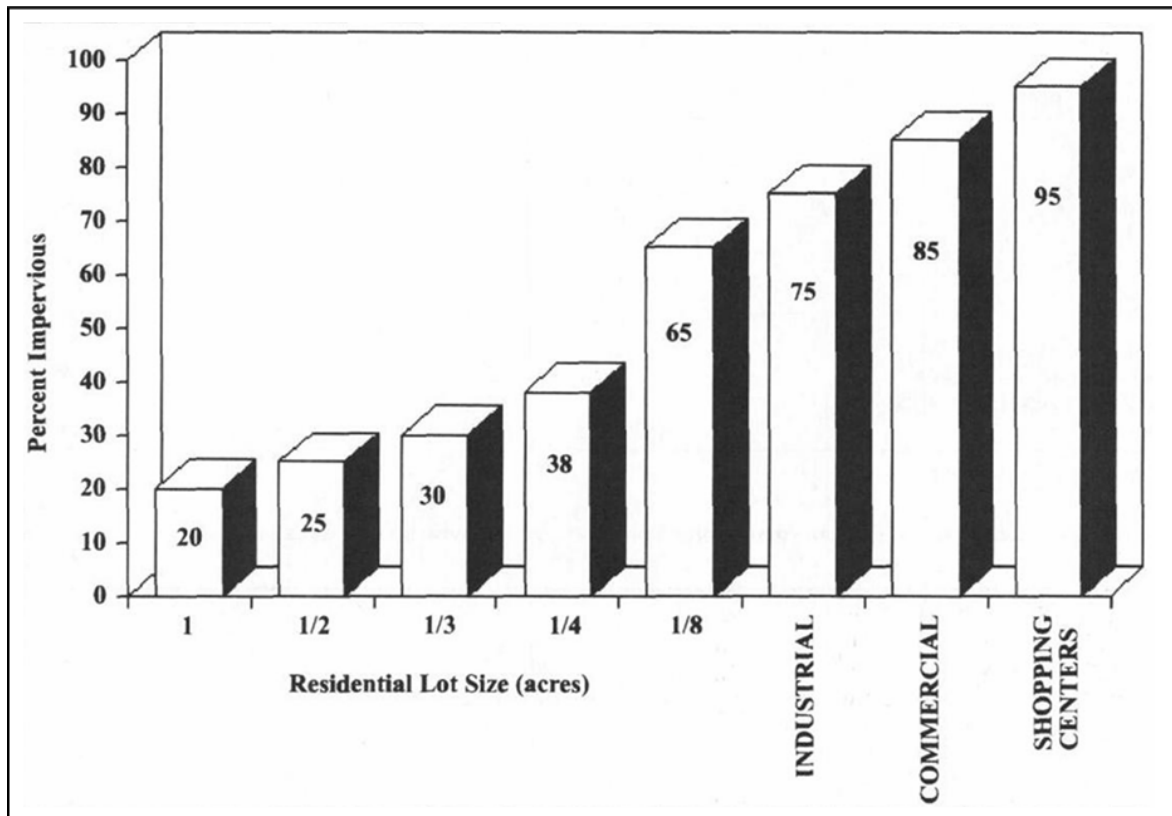


Figure 1.1. Average percent impervious cover by increasing density of urban land use. Taken from (Arnold & Gibbons 1996).

A second major source of urban pollution in water ways is sewage. The direct release of treated sewage and the inadvertent seepage of groundwater borne sewage from septic tanks and leaking sewer lines conveys additional nutrient loads to urbanised waterways (Valiela et al. 1997, Walsh 2000, Steffy & Kilham 2004). However, in areas with adequate sewage systems, urban runoff is considered the primary cause of degradation (Walsh 2000, CWP 2003). Due to the strong relationship between IC and the volume of urban runoff entering waterways, IC is becoming a widely accepted indicator of urban impact (Arnold & Gibbons 1996, CWP 2003). Water quality generally starts to decrease when IC exceeds 10%,

and obvious reductions in biotic health occur when more than 10 – 25% of the catchment is converted to impervious surfaces (e.g. Arnold & Gibbons 1996, CWP 2003, Walsh 2004). Studies examining the relationship between IC and receiving water quality have shown strong associations between IC and levels of hydrocarbons, pesticides, metals, nutrients and faecal coliform bacteria (e.g. Mallin et al. 2000, Van Dolah et al. 2008). Negative relationships between IC and various responses in biota have been well documented for freshwater environments (e.g. Arnold & Gibbons 1996, Walsh et al. 2001, CWP 2003). However, few studies have examined this relationship in estuaries. Lerberg et al. (2000) and Holland et al. (2004) documented notable biotic responses to urbanisation in tidal creeks around South Carolina, USA, when IC exceeded 20 – 30 %, including a reduced abundance of stress-sensitive macrobenthic taxa and altered food webs. However, these systems had macro-tidal ranges of 4.6 – 7.9 m and vegetation dominated by marsh grass (Van Dolah et al. 2008), making the applicability of these results to less flushed estuaries with smaller tidal ranges uncertain.

Due to their large spatial scale, relationships between IC and ecosystem degradation are likely to be susceptible to variability from natural parameters such as the size of the water way of interest, flow rates and the extent of tidal flushing. The presence of additional anthropogenic factors such as the point-source discharge of sewage may also influence the relationship. Where possible, these potentially confounding influences should be accounted for in the experimental design, statistical analysis and interpretation of the relationship.

## **1.5 Urban pollutants: a cocktail of counteractive effects**

Urbanisation delivers a cocktail of pollutants to estuaries, including nutrients, sediments, metals, petrochemical hydrocarbons, and increased fresh water flows (Drapper et al. 2000, Valiela & Bowen 2002, CWP 2003). Sources of N include groundwater inputs from septic tank systems, discharged treated sewage, excavated soil at construction sites, lawn fertilisers and animal wastes (Valiela et al. 1997, Carpenter et al. 1998, CWP 2003). Urban nutrients may also enter estuaries from neighbouring parks, golf courses and artificial water features which have been irrigated with recycled wastewater (tertiary treated effluent) (EPA 2005b). The increased input of N into estuaries has become a serious environmental issue. Slight elevations in N loads may initially subsidise an ecosystem, increasing algal growth to a level that also enhances the growth and condition of consumers. Excessive N loads, however, have a stress effect on ecosystems, stimulating algal growth until primary

production exceeds the metabolism of organic matter, resulting in hypoxic conditions and ecosystem degradation (Odum et al. 1979, Nixon 1995, Cloern 2001).

Urbanisation is also linked with increased sedimentation of estuaries (Anderson et al. 2004). The predominant sources of sediments in urbanised catchments include erosion from exposed soils such as construction sites, wash off from sediment accumulated on impervious areas, and increased stream bank erosion caused by increased stormwater runoff volumes (CWP 2003). The fine terrigenous sediments delivered in urban runoff have slow settling rates increasing the mean turbidity of the water column (Schueler 1987, MacIntyre et al. 1996, CWP 2003, McLusky & Elliott 2004). These increases in turbidity reduce light penetration to the epibenthos, which can in turn reduce primary production (MacIntyre et al. 1996). The deposition of fine terrestrial sediments has also been linked to changes in estuarine macrofauna assemblages (e.g., Edgar & Barrett 2000, Anderson et al. 2004).

Sediments convey other urban pollutants such as metals, hydrocarbons and nutrients, which can bind to sediment particles (CWP 2003). These pollutants flow into coastal waterways in urban runoff, reducing water quality and degrading habitats (Costanza et al. 1998, Drapper et al. 2000, Lee et al. 2002). Mangrove-dominated estuaries typically have fine, muddy sediments with high organic content. Pollutants such as metals and pesticides bind readily to these sediments, making these environments susceptible to the accumulation of urban pollutants (e.g., Abraham et al. 2007). A portion of these pollutants can accumulate in the tissues of estuarine organisms and biomagnify through the food web (Connell et al. 1999, Barwick & Maher 2003). Exposure to these pollutants can lead to sublethal or lethal effects in biota potentially leading to changes in community composition (Lerberg et al. 2000, Leung & Furness 2001, Moreno-Garrido et al. 2003, Elumalai et al. 2005, McGill et al. 2007). The components of urban runoff may also behave counteractively or synergistically with each other (Figure 1.2). For example, small increases in N loads, which promote primary production, may be offset by increased sediment loads which suppress primary production. The salinity changes associated with greater stormwater loads can also lead to sublethal effects in biota (Morritt et al. 2007).

The cocktail of urban pollutants entering estuaries can degrade ecosystem health by altering the abundance of nutrients and their source, enhancing or suppressing primary production, and altering the condition of animals or the structure of the assemblage (Holland et al. 2004).



## 1.6 Ecosystem health

In response to the increasing evidence that human-dominated ecosystems are becoming dysfunctional, a concept of ecosystem health was developed by Rapport et al. (1979). The term ‘ecosystem health’ is the state, condition or performance of an ecosystem, as defined by a predetermined benchmark or reference value (Rapport et al. 1998, Fairweather 1999). This benchmark is based upon our perception of a healthy ecosystem which is typically based upon the ecosystem’s capacity to provide services that benefit humankind. Therefore, our concept of ecosystem health is often subjective, based upon our ideas of what an ecosystem should be like and our attitudes towards nature (Depledge 1994, Fairweather 1999). Due to this subjectivity, the definition of ecosystem health remains a topic of ongoing debate amongst scientists (Karr 1993, Wicklum & Davies 1995, Rapport 1998a, Vugteveen et al. 2006). This, itself may be viewed as healthy as it is likely to make our concept of ecosystem health more objective. In any case, the term ecosystem health is a powerful communication tool for spreading ecological knowledge from scientists to the wider public and political leaders (Fairweather 1999, Vugteveen et al. 2006). This is pivotal in ensuring that ecological monitoring has any influence on environmental policies and on the mitigation of human disturbances to ecosystems (Karr 1999).

Similar to human health, ecosystem health is assessed using a number of metrics. For example a human health practitioner may gauge a person’s health by checking their blood pressure, body mass index and core temperature. Ecosystem health is assessed using measures of organisation, vigour and resilience (Rapport et al. 1998, Fairweather 1999, Vugteveen et al. 2006, Thrush et al. 2008). Organisation is measured as the diversity and number of ecosystem components, and their interactions, for example, species composition or food web structure. The functionality of ecosystems is strongly influenced by their biodiversity. The more functional groups there are in an ecosystem, the greater its capacity to deliver ecosystem services (Luck et al. 2003). The more species there are in each functional group, the more resilient an ecosystem is likely to be due to a greater chance of “redundant” species surviving future disturbance and filling the functional role of more sensitive species (Walker 1995, Ehrlich & Walker 1998). Resilience is defined as an ecosystem’s capacity to recover from disturbance and maintain its structure and function (state) in the presence of stress (Rapport et al. 1998). When an ecosystem’s resilience is exceeded, it may switch to an ‘alternate’, or degraded state (Rapport et al. 1998, Scheffer et al. 2001). Resilience can be measured as the largest disturbance from which an ecosystem can ‘recover’ (Rapport et al. 1998). Although, recent studies show that an ecosystem’s rate of recovery may also offer a

good indicator of resilience (Thrush et al. 2008). Recovery is considered as the return of an ecosystem to its previous state before a disturbance. An ecosystem's potential to recover and hence its resilience may also be influenced by physical and ecological factors, such as, sediment composition and ecological connectivity (Thrush et al. 2008). Therefore it is important to consider these factors when measuring resilience. Vigour is an ecosystem's rate of activity, which can be assessed as the rate of various ecosystem processes such as primary production, competition, herbivory and decomposition (Rapport et al. 1998, Fairweather 1999).

There remains some uncertainty as to what represents 'good' health for many ecosystems (Fairweather 1999). Therefore, signs of stress in ecosystems are also measured to assess ecosystem health. The Ecosystem Distress Syndrome (EDS) is a concept developed by Rapport et al. (1998b) to describe common patterns of degradation associated with stressed ecosystems. These patterns include biotic impoverishment, altered nutrient cycling, eutrophication, an increase in the ratio of *r*-selected to *K*-selected species and decreased resilience (Rapport 1992, Rapport 1998b). The health of an ecosystem is considered to be degraded when signs of EDS are present. Although useful, many of these indicators signal an impact when the ecosystem has already changed to, or has started to change to a degraded state.

As ecosystems move towards altered or degraded states, feedback loops can occur which maintain the ecosystem in the alternative state, making it difficult to restore the ecosystem to its prior state (Scheffer & Carpenter 2003, Lee et al. 2006). For example, feedbacks between oxygen dynamics and N cycling exist; hypoxic conditions tend to inhibit nitrification, reducing the transformation of ammonium to nitrate. This in turn reduces the denitrification of nitrate into N gas, reducing the loss of N from the system to denitrification (Kemp et al. 1990, Cloern 2001). Therefore, when an ecosystem starts to become eutrophic, this feed back mechanism increases the efficiency of N retention, pulling the ecosystem further towards a eutrophic state. Such shifts in state can cause catastrophic changes to the structure and function of ecosystems, and to the services that they provide. Failure to predict and prevent these changes to degraded states may be costly to society (Carpenter et al. 1999, Scheffer et al. 2001, Beisner et al. 2003).

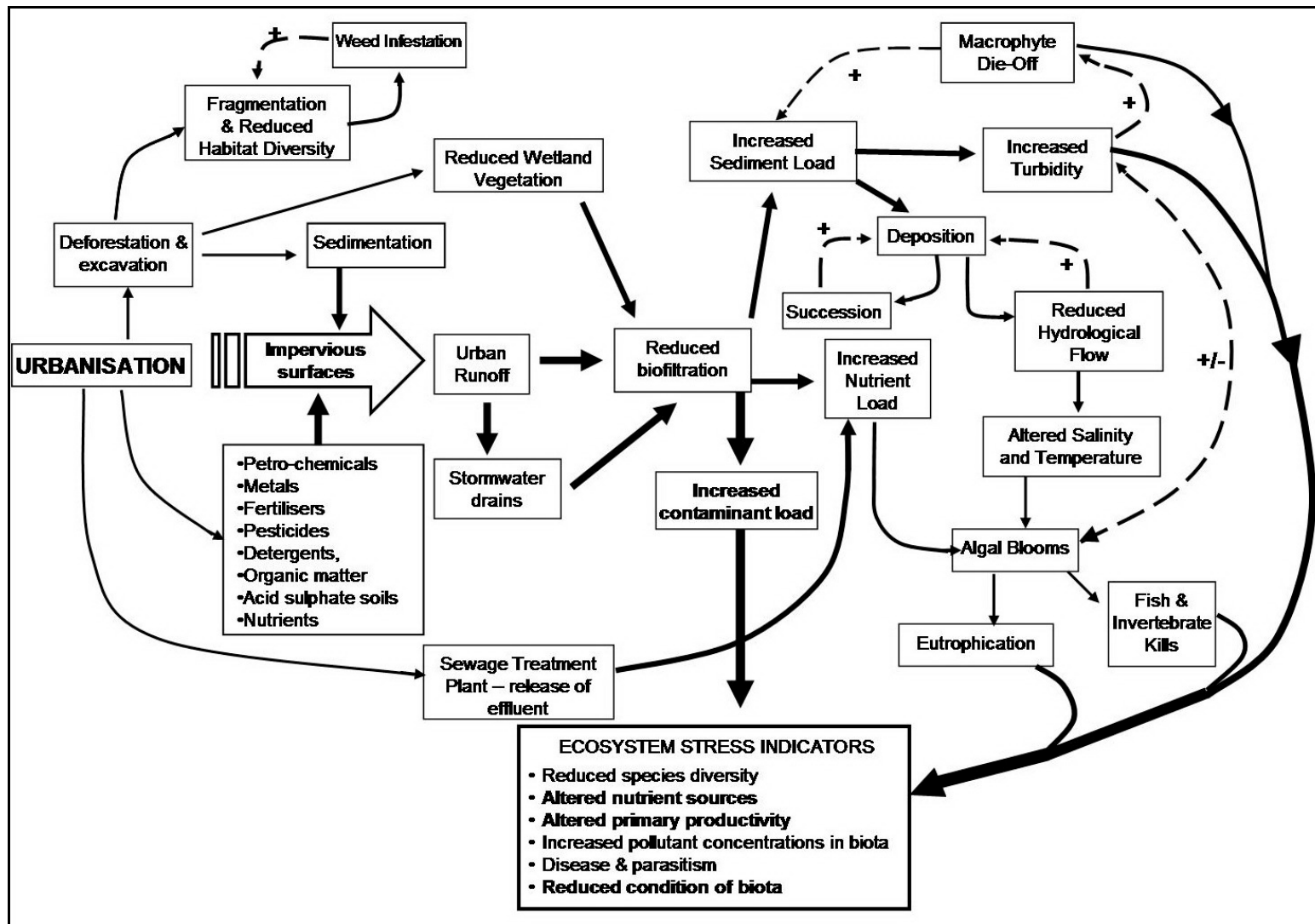


Figure 1.2. A summary of some of the impacts urbanisation may have on the ecosystem health of estuaries showing counteractive and synergistic interactions likely to occur between pollutants, as well as feedback loops which may maintain an ecosystem in a degraded state. Modified from Lee et al. (2006).

### 1.6.1 Biotic indicators of ecosystem health

Restoration of degraded ecosystems and of the services they provide often requires radical and expensive intervention and may not always be possible (Maler 2000, Rapport 2002). Many environmental managers rely upon conventional measures such as species diversity to monitor ecosystem health. Generally a high ratio of tolerant to sensitive species indicates that an impact has occurred. In cases where the natural history of all species is understood, such changes in diversity may be useful in providing an indication of the functionality of an ecosystem (Ehrlich & Walker 1998). However, decreases in species diversity often signify a reduction in the number of functional groups within an ecosystem, or at least a reduction in the number of species within each functional group, thus indicating that the ecosystem's capacity to function and provide services has already reduced (Walker 1995, Ehrlich & Walker 1998). Therefore, declines in species diversity signify an impact when an ecosystem has already become degraded.

Logically, any indicator of ecosystem health is only capable of detecting an impact when some degree of degradation has occurred. However indicators which are able to detect signs of stress at a stage when degradation to the ecosystem is minimal, i.e. before declines in species diversity have occurred, are likely to make remediation more achievable. Indicators measured in biota offer a way to detect signs of stress early. In particular, indicators that are linked with declines in the fitness or survival of ecologically important taxa may enable the early detection of ecosystem degradation (Depledge & Fossi 1994). These types of indicators are likely to be more cost effective than more traditional measures such as species diversity as they may enable the mitigation of impacts before the ecosystem's capacity to provide valuable services has been substantially reduced.

Unlike conventional measures of water quality and pollutant concentrations, the *in situ* measurement of biotic indicators captures the long term responses of biota to subtle increases in pollutant loads, as well as the synergistic and counteractive responses to different pollutants occurring simultaneously in the environment. Biotic indicators also reflect the toxicity of pollutants under naturally variable conditions (e.g. salinity and temperature) as well as the indirect effects that pollutants may have on biota due to their impacts on species interactions (Phillips & Rainbow 1993, Depledge 1994, Handy et al. 2003). Therefore, the *in situ* measurement of biotic indicators offers a more sensitive method for the early detection and mitigation of urban pollutants, thus improving the likelihood of preventing ecosystem degradation.

Bayne et al. (1985), Phillips & Rainbow (1993), Fairweather (1999), and Hilty & Merenlender (2000) recommend that indicators match the following criteria to ensure they provide reliable estimates of the condition of their ecosystems:

- Relationships should exist between the indicators and the pollutants associated with ecosystem degradation,
- Indicator responses should have ecological significance and be linked to adverse effects on the survival of taxa which contribute significantly to the structure and function of the ecosystems at risk,
- Responses should not alter significantly with short-term variations in pollutant load – if they do this should be incorporated into the design of the monitoring experiments,
- The response of indicators should be easily detectable above natural variability and have a high signal to noise ratio and,
- Indicators should be easy to measure and replicate through time and at multiple sites without the need for, complicated procedures or high running costs.

Furthermore, the biota in which the responses are measured should be representative of the study site (Fairweather 1991, Hilty & Merenlender 2000). Therefore animals that are measured need to be relatively non-mobile or at least have a home range that reflects the spatial scale of the monitoring study.

#### ***1.6.1.1 Composite indicators***

Biotic indicators which can detect alterations to nutrient sources, or changes in the biomass and condition of ecologically important taxa are likely to enable detection of impacts before the structure and functionality (e.g. biodiversity and productivity) of an ecosystem has become significantly degraded. The combination of such indicators into a composite index of health (i.e. the condition of biota) offers a single measure of the multi-trophic responses to disturbance and therefore a better estimate of response at the ecosystem level. Examples of composite indices include an index used by the Queensland EPA to monitor the health of southeast Queensland's estuaries. This index combines various water quality measures, including nutrient, turbidity and chlorophyll *a* (Chl *a*) levels, to provide an index of health for estuaries (EPA 2007). Other indices combine a broader variety of measures such as, habitat quality, the species richness of fish, the number of dominant species and, trophic functional groups (e.g., Bilkovic & Roggero 2008). In addition to providing a better indication of the ecosystem level responses to disturbance, composite

indices improve the ease of communicating ecological responses to environmental managers and policy makers (Galloway et al. 2004, Bilkovic & Roggero 2008).

#### ***1.6.1.2 Nitrogen stable isotopes as tracers of urban N***

Excessive nutrient loading from urban land uses can lead to eutrophication in estuaries (e.g., Mallin et al. 2004, Kemp et al. 2005). Eutrophication is typically defined as an increase in the supply rate of organic matter (Nixon 1995). This description assumes a proportional response in algal growth per unit increase in nutrient loading. In estuaries, however, this response is far more complex due to a variety of system-specific features that may filter the effects of N loading (Cloern 2001). In this thesis eutrophication is defined as excessive nutrient loading (N or P, phosphorus) that stimulates algal production beyond a systems capacity to metabolise it aerobically, resulting in the microbial breakdown of algae and subsequent bottom water hypoxia (Cloern 2001).

Ratios of nitrogen stable isotopes (measured by  $\delta^{15}\text{N}$ ) offer a useful tracer for isotopically distinct N sources including synthetic fertilisers, which typically have  $\delta^{15}\text{N}$  values between  $-8$  and  $7\text{‰}$ , and urban sewage, which has  $\delta^{15}\text{N}$  values between  $10$  and  $22\text{‰}$  (Macko & Ostrom 1994, Figure 1.2). Therefore,  $\delta^{15}\text{N}$  values provide a more accurate means of identifying N sources in estuaries compared to more conventional measures of N concentration in the water column. Furthermore,  $\delta^{15}\text{N}$  values can be obtained from small amounts of biotic tissue ( $< 5$  mg, dry weight) and samples require little preparation prior to measurement in a mass spectrometer. Therefore, the measurement of  $\delta^{15}\text{N}$  in estuarine biota offers a cost effective means of detecting and mitigating urban N loads to estuaries.

The  $\delta^{15}\text{N}$  values of estuarine biota can detect urban N in estuaries at relatively low loading rates with  $\delta^{15}\text{N}$  values in estuarine biota becoming progressively enriched with greater urban N loading and with increased proximity to urban N sources (McClelland & Valiela 1998, Costanzo et al. 2001, Moore & Suthers 2005, Savage 2005, Schlacher et al. 2005, Dillon & Chanton 2008). Therefore the  $\delta^{15}\text{N}$  of estuarine biota may provide an early warning for potential eutrophication in urbanised estuaries (McClelland & Valiela 1998). The  $\delta^{15}\text{N}$  values of estuarine biota exposed to N derived from urban sewage are typically more enriched than that of biota that assimilate N from natural sources (Tucker et al. 1999, Savage & Elmgren 2004, Schlacher et al. 2005). The tertiary treatment of urban sewage elevates the  $\delta^{15}\text{N}$  of wastewater N above background values, through nitrification and denitrification processes (Heaton 1986). Similarly, groundwater sources of wastewater-derived N from

septic tanks and leaking sewage lines become enriched in  $^{15}\text{N}$  as ammonia volatilisation and denitrification processes remove  $^{14}\text{N}$  more rapidly than  $^{15}\text{N}$ , leaving the remaining nitrate isotopically enriched (Macko & Ostrom 1994, McClelland & Valiela 1998). Recently it has been discovered that N in urban runoff is also isotopically enriched, due to the thermodynamically favoured volatilisation of  $^{14}\text{NH}_3$  from stormwater as it flows across warm impervious surfaces (Dillon & Chanton 2005). Therefore the  $\delta^{15}\text{N}$  of estuarine biota may also provide a convenient tracer for the increased conveyance of N in urban stormwater into estuaries via impervious surfaces. Frequent monitoring of  $\delta^{15}\text{N}$  in biota may enable the early detection of urban N in estuaries, which may lead to eutrophication and ecosystem degradation in urbanised estuaries (McClelland et al. 1997).

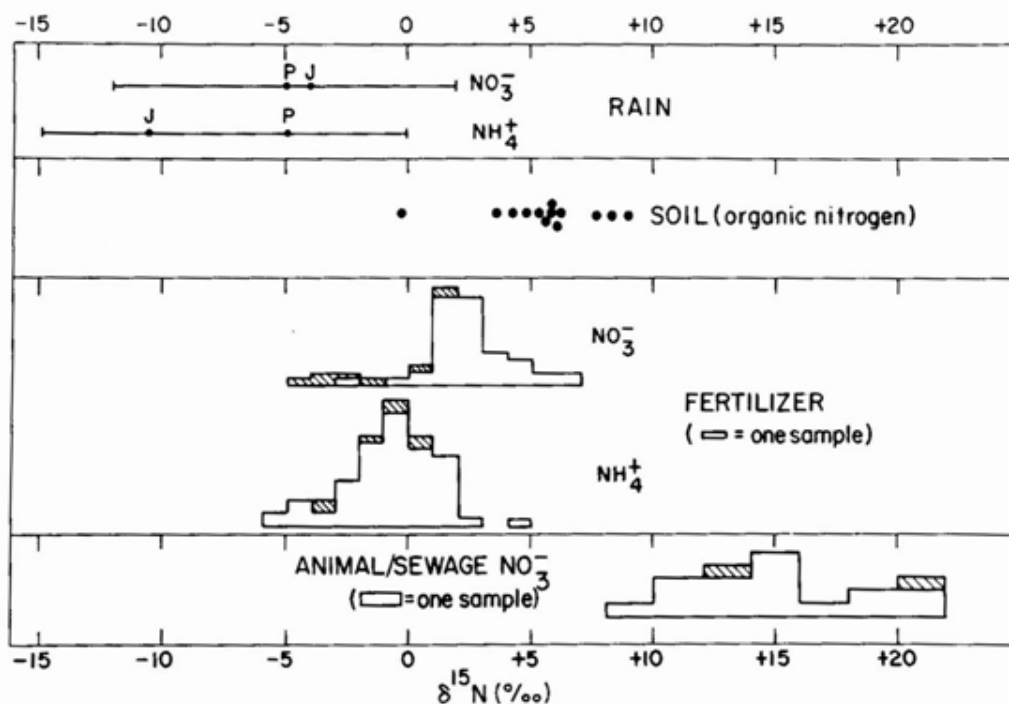


Figure 1.3. Summary of the range of observed  $\delta^{15}\text{N}$  values for the major potential sources of nitrogen (N) in ground and surface waters. Copied from Heaton (1986).

### 1.6.1.3 *Microphytobenthos biomass*

The intertidal flats of estuaries are covered by mobile epipellic diatoms and cyanobacteria collectively known as microphytobenthos (MPB). Crabs in mangrove forests are often seen grazing on surface sediments, which are likely to contain MPB (Micheli 1993) and studies using stable isotopes as dietary tracers indicate that MPB are an important energy source to many estuarine consumers including crabs (Dittel et al. 1997, France 1998, Lee 2000, Bouillon et al. 2002a, Bouillon et al. 2002b, Guest et al. 2004, Bouillon et al. 2008).

MPB is also likely to sustain pelagic consumers when it is resuspended into the water column during tidal inundation and/or wind-induced turbulence in shallow embayments (De Jonge & Van Beuselom 1992, Lucas et al. 2001). MPB also contribute to biogeochemical processes in estuaries, and thus play an important role in sediment carbon and nutrient cycling and exchange with the water column (Baillie 1986). The extracellular polymeric substances (EPS) produced by MPB also provide an important carbon source for bacteria and grazers, and may help to stabilise surface sediments, potentially reducing erosion of the benthos (Wolfstein & Stal 2002).

Urban pollutants can alter the productivity of MPB. Slight elevations in N loading are typically associated with increased MPB growth, whereas excessive N loading can lead to eutrophication and declines in MPB biomass due to bottom water hypoxia and shading associated with increased phytoplankton biomass (Cadée 1984, Meyercordt & Meyer-Reil 1999, Lever & Valiela 2005, Yoshiyama & Sharp 2006). Increased exposure to other urban pollutants such as, sediments, herbicides and metals can also reduce MPB production (Admiraal & Peletier 1980, McIntyre & Flecker 2006). Toxicity tests have shown dose dependent declines in the survival of benthic diatoms exposed to increased concentrations of metals (Moreno-Garrido et al. 2003).

Due to the trophic importance of MPB, monitoring of its responses to increasing urbanisation is important, as impacts on its productivity are likely to cascade throughout the estuarine food web. Monitoring of MPB production, together with the  $\delta^{15}\text{N}$  of biota may provide early indication as to whether increased urban N inputs are contributing to increased algal growth and eutrophication in estuaries. Furthermore, the productivity of MPB can be estimated using a number of relatively simple, low cost methods, including the spectrophotometric determination of chlorophyll pigments, *in situ* changes in  $\text{O}_2$  and  $\text{CO}_2$  concentrations and uptake rates of  $^{14}\text{C}$  (Parsons et al. 1984, Blanchard et al. 1996, Barranguet et al. 1998, Lassen et al. 1998, Spilmont et al. 2006).

#### **1.6.1.4 Consumer condition**

The measurement of condition in estuarine consumers is likely to provide an early warning for changes in ecosystem health, particularly in biota that have a substantial influence on the structure and functioning of ecosystems (Chapin III et al. 1997). Brachyuran crabs are the most widespread and abundant consumers of the intertidal mangrove flats and contribute importantly to the structure and function of mangrove ecosystems (Snelling 1959, Tan & Ng 1994, Hartnoll et al. 2002, Skov et al. 2002, Lee 2008). Therefore, early detection



of reductions in the condition of these taxa can increase the likelihood of managers being able to reverse urban impacts before estuarine ecosystems change to a degraded state.

In crustaceans, the hepatopancreas stores nutrients, metabolises energy reserves and removes toxins (Kennish 1997, Connell et al. 1999, Verri et al. 2001). The ratio of the hepatopancreas mass to body mass is referred to as the Hepatosomatic Index (HI), a convenient, simple to measure indicator of body condition in crabs and other invertebrates. The relative size of the hepatopancreas can provide an indication of the state of an animal's habitat as it responds to changes in the energy demands of the animal (Phillips & Rainbow 1993). For example, the condition (HI) of herbivorous crabs would increase when nutrient loads result in an increase in algal food availability (Kennish 1997). Whereas, exposure to pollutants such as heavy metals has been reported to cause dose dependent reductions in the HI of crabs (Elumalai et al. 2005). Chaufan et al. (2006) reported a 20% reduction in the HI of estuary crabs that absorbed the hydrocarbon hexachlorobenzene through the ingestion of algae exposed to the toxin, indicating a cascade of impact between primary producers and consumers.

The measurement of crab condition and of the productivity of their algal food sources (MPB) is likely to provide an indication of the multi-trophic responses to urban pollutants. Crab condition may also offer an indication of the animal's resilience to additional impacts as crabs with reduced body condition are likely to have fewer nutrient reserves for repair and maintenance (e.g., Dissanayake et al. 2008). Such reductions in the mean condition of crab populations may lead to decreases in their survival when confronted with additional disturbance (Depledge 1994). Any decline in the population of these important ecosystem engineers is likely to alter the structure and function of mangrove ecosystems, in turn, reducing their resilience to additional disturbance. Therefore, by providing an indication of changes in crab condition before declines in local crab populations occur, the HI offers a cost effective means of detecting impacts at a stage when there is a greater chance of remediation.

#### ***1.6.1.5 Temporal variance: a method of detecting antagonistic responses and reduced resilience?***

Urbanisation delivers a cocktail of pollutants to estuarine environments and these pollutants can have opposing effects on biota. Such counteractive effects may mask any net long term change in biota and hence prevent an impact from being detected. Different types of urban pollution, however, tend to enter estuaries over different time scales. For example, the daily discharge of N from a treated sewage outflow compared to occasional pulses of

sediment in urban stormwater. Primary production and consumer condition are initially likely to increase when urban N is the most prevalent pollutant, but these quantities would probably decrease at times when urban stormwater delivers stressors such as sediments or heavy metals to an estuary. These changes through time are likely to be more pronounced in estuaries exposed to greater sewage and runoff loads. Therefore, measurement of the temporal variability in indicator responses may provide a better means of detecting urban impacts in estuaries, rather than simply trying to detect any net change in long-term indicator values (Underwood 1998).

This type of stress is important to detect early because more variable ecosystems, oscillating from one extreme to another, are more likely to cross thresholds and switch to degraded states (Underwood 1998, Litzow et al. 2008). For example, during periods of exposure to stormwater toxicants reductions in the abundance of benthic algae and grazers may occur. However, when nutrient loading is increased, primary production is likely to increase, but due to the reduction in grazers, the ecosystem's capacity to control the increased algal growth is reduced. Therefore, the resilience of the ecosystem to nutrient loading has been decreased and the risk of changing to a eutrophic state is greater. Recent studies propose the use of ecosystem variability as an indicator of resilience with greater variability signifying a reduction in resilience and an increased risk of switching to an altered state (Scheffer & Carpenter 2003, Hughes et al. 2005, Oborny et al. 2005, Brock & Carpenter 2006, Litzow et al. 2008).

## **1.7 Urbanisation of southeast Queensland and overall rationale of the thesis**

The coastline of southeast Queensland, Australia, encompasses a diverse array of estuarine environments. These range between large coastal embayments and small tidal creeks, and contain extensive mangrove, saltmarsh, seagrass, mudflat and sandflat habitats. Many species of invertebrates, fish, birds, reptiles and mammals depend on these ecosystems for nursery habitats and feeding grounds (Lee 1999). The ecological significance of these environments is recognized in that much of the regions coastline is protected as part of the Moreton Bay Marine Park. Southeast Queensland is, however, one of the fastest growing regions in Australia with its human population projected to increase from the present 2.9 to ~ 4 million by 2026 (OUM 2004, DIP 2008). Rapid urbanisation of the region's coastal areas is already impacting the water quality of estuaries in the region (EPA 2007).

While the Queensland Environmental Protection Agency (EPA) and local catchment groups monitor the water quality of estuaries in the region, I am not aware of any investigation that has specifically assessed the biotic or ecosystem level responses of estuaries to increasing urbanisation. An understanding of these responses is essential for the sustainable management of urbanisation in the region. Evidence from studies in tidal creeks in South Carolina, USA, indicate that the health of estuaries is inversely related to urbanisation, measured as IC (Lerberg et al. 2000, Holland et al. 2004). These systems, however, differ substantially from the estuaries of southeast Queensland, due to their larger tidal ranges (4.6 – 7.9 m against < 2.0 in southeast Queensland) and intertidal vegetation dominated by marsh grass rather than mangroves (Van Dolah et al. 2008). Therefore, the relationship between urbanisation and the biotic health of estuaries in southeast Queensland may differ from that observed in these systems. Furthermore, these studies assessed the relationship between urbanisation and measures of community composition, a measure that detects degradation only when the diversity or organisation of the ecosystem has already changed – already degraded environments are difficult and costly to restore.

Assessment of this relationship using biotic indicators which are capable of detecting impacts before the health of an ecosystem degrades provides an opportunity for managers to mitigate urban pollution while its impacts can still be reversed. Indicators that signal changes to nutrient sources and changes in the condition of biota that contribute significantly to the structure and function of an ecosystem are likely to be very useful in this regard. This thesis investigates whether urban growth around coastal southeast Queensland is impacting the biota of estuaries around the region. A suite of indicators which are likely to signal early signs of degradation were measured in mangrove biota. These indicators included an isotopic tracer for altered nutrient sources, benthic algal biomass (as a proxy for benthic primary production), and a condition index in functionally significant estuarine consumers.

## **1.8 Structure of the thesis**

The remainder of this thesis is presented as a series of chapters testing the responses of biotic indicators to urbanisation in mangrove-dominated estuarine environments around southeast Queensland. Studies were conducted at various spatial and temporal scales and in different types of estuaries typical of subtropical southeast Queensland, in order to investigate the scale and nature of urban impacts in the region's estuarine environments.

**Chapter 2** presents a case study of Lake Coombabah, a moderately urbanised estuarine lake in southeast Queensland. This study tested whether the mean and temporal

variation of  $\delta^{15}\text{N}$  in the grey mangrove, *Avicennia marina*, and a common ocypodid crab, *Australoplax tridentata* (tuxedo crab), the biomass of MPB and condition of crabs changed with proximity to three urban influx sites around the lake. These local scale responses to urbanisation were measured three times during the wet and dry seasons of 2005 – 2006.

**Chapter 3** presents a study which measured the responses of the biotic indicators reported in Chapter 2 to increasing levels of urbanisation surrounding five estuarine lakes. In this study I measured the mean and the temporal variability of the biotic indicators at the ‘whole’-lake level over a period of one year. I tested the hypothesis that these metrics would indicate a decline in the biotic condition of the lakes with increased urbanisation (measured as the IC of the surrounding catchments).

**Chapter 4** reports an investigation of the relationship between the biotic indicators and urbanisation in eight mangrove-lined estuarine creeks. The presence of tertiary-treated sewage discharge was also tested as a potential factor affecting the health of biota in the creeks. I tested the hypotheses that the mean  $\delta^{15}\text{N}$  in mangroves and crabs, MPB biomass, and crab condition would indicate a decline in the biotic condition of the creeks with increased urbanisation (IC); and that the mean  $\delta^{15}\text{N}$  in mangroves and crabs would be more enriched in creeks exposed to sewage effluent discharge.

**Chapter 5** describes a laboratory experiment using mesocosms to simulate natural conditions, examining the independent and interactive effects of increased sediment and nutrient inputs on MPB biomass and crab condition. I tested the hypothesis that the sediment additions would have a greater effect on the indicators than the addition of N.

**Chapter 6** provides a summary of the main findings of the thesis and discusses their implications in terms of the future monitoring and management of estuarine health responses to urbanisation.



## Chapter 2. Local effects of urban influx from sub-catchments of Lake Coombabah

### 2.1 Abstract

Urbanisation generates a cocktail of pollutants which are conveyed across impervious surfaces into estuaries via stormwater drains and creeks. Indicators which offer early detection of impact can assist in preventing these pollutants from degrading the health of estuaries. This chapter investigated the localised responses of mangrove biota to urban pollution in Lake Coombabah, located on southeast Queensland's rapidly urbanising Gold Coast. Most of the urban pollution in the system is likely to enter the lake via two creeks (East Creek, EC, and Coombabah Creek, CC) and a stormwater pipe which drains the Helensvale (HV) residential estate. Therefore, I compared responses in biota located near (within a vicinity of 10 m) these three influx sites with the responses in biota located farther away (250 m from the influx sites).

Stable nitrogen (N) isotope ratios ( $\delta^{15}\text{N}$ ) were measured in the leaves of the grey mangrove (*Avicennia marina*) and in the hepatopancreas and muscle tissues of the tuxedo crab (*Australoplax tridentata*) to provide an indication for the presence of urban N sources. The biomass of benthic microalgae (microphytobenthos, MPB) and the relative hepatopancreas weight of crabs were measured as indicators of benthic primary productivity and crab condition. These indicators were measured on six occasions during September 2005 – January 2006. I predicted that biota living close to the influx sites would have more enriched mean  $\delta^{15}\text{N}$  values than biota living farther away, due to a greater assimilation of urban N sources, and that benthic primary production and crab condition would differ between biota living near and far the urban influx sites. I also predicted that these indicators would vary more through time in biota near the influx sites due to greater exposure to (more concentrated) pulses of urban runoff delivered during times of rainfall.

Enrichment of mangrove and crab  $\delta^{15}\text{N}$  values ( $4.5 \pm 0.1$  and  $6.8 \pm 0.1\text{‰}$ ) at the EC site, which is adjacent to a sewage treatment plant (STP), indicated that N from treated sewage is assimilated by biota at this site. An increase in the temporal variability of crab condition near this site (Coefficient of variation:  $28.4 \pm 6.0\%$ ), indicated that the assimilation of sewage N and exposure to urban runoff is having a localised impact on the condition of crabs near this site. Following rainfall,  $\delta^{15}\text{N}$  values became enriched in crabs both near and

far from the CC influx site and at the HV stormwater drain indicating that urban stormwater is a source of urban N at these two sites.

As well as indicating the sources of urban pollution to the lake, these responses display signs of altered nutrient sources and a localised impact on the condition of biota in the lake, and thus provide an early warning signal for ecosystem degradation. The localised scale of these impacts may be useful in providing managers with clues of how to mitigate further urban flows into the lake.

## 2.2 Introduction

Estuaries provide vital ecosystem services. These include the storage and filtration of flood waters, the assimilation of sunlight and nutrients into food webs, and essential habitats to many fish, invertebrate and bird species (Lee 1999). However, the capacity for estuaries to provide these services is at risk due to rapid urbanisation of coastal areas. Today approximately 60% of the human population live within 100 km of the coast (Vitousek et al. 1997). Landscape changes associated with urbanisation, such as the replacement of forest cover with impervious surfaces, lead to the increased runoff of urban pollutants into coastal waterways (Arnold & Gibbons 1996, Wissmar et al. 2004). Many urban pollutants originate from diffuse non-point sources, for example, sediments and metals from road runoff (Drapper et al. 2000, Barry et al. 2004). Following rainfall, impervious surfaces channel the flow of these pollutants into estuaries, where they are often delivered as a point source discharge via stormwater pipes, creeks and other influx points (Sanger et al. 1999a, Hatt et al. 2004, Van Dolah et al. 2008). Urbanisation also leads to an influx of wastewater nitrogen (N) sources into groundwater, which can occur as a result of poorly maintained septic tanks and leakage of effluent from damaged sewer lines (Valiela et al. 1997, Steffy & Kilham 2004). Impervious surfaces, adjacent to parks which have been irrigated with recycled wastewater may also convey sewage N into waterways (EPA 2005b). These groundwater and surface runoff sources of urban N can flow into estuaries via creeks and other surface waters (Downing & Peterka 1978, McClelland & Valiela 1998).

Urbanisation can degrade the ecosystem health of estuaries. The influx of urban pollutants can lead to changes in system organisation (e.g. biodiversity), vigour (e.g. respiration or primary productivity), and ecosystem resilience (counteractive capacity) (Rapport 1998b, Holland et al. 2004). Restoration of already degraded ecosystems is complex, so there is a need for indicators that can detect impacts early, preferably before the structure (e.g. biodiversity) or the functionality (e.g. nutrient cycling) of an ecosystem has changed. Although not a direct measure of ecosystem health, biotic measures, such as pollutant tracers and condition indices in ecologically significant taxa can offer early indication of impacts to ecosystem health.

The  $\delta^{15}\text{N}$  values of biota provide a convenient tracer for the sources and fates of nutrients in food webs (Michener & Schell 1994) and in recent years,  $\delta^{15}\text{N}$  has been used to trace the assimilation of isotopically enriched urban sources of N in estuarine food webs (McClelland & Valiela 1998, Costanzo et al. 2001, Savage & Elmgren 2004, Dillon &



Chanton 2008). When combined with estimates of primary productivity, the measurement of  $\delta^{15}\text{N}$  in estuarine biota may extend our understanding of how estuarine ecosystems respond to urban nutrient loads.

Measures of condition in estuarine consumers are also likely to provide early indication of impacts, before changes in species composition occur. In crustaceans, the hepatopancreas is an organ involved in the storage of nutrients, metabolism of energy reserves and removal of toxins (Kennish 1997, Connell et al. 1999, Verri et al. 2001). The ratio of hepatopancreas mass to body mass (Hepatosomatic Index, HI (%)) is used as an indicator of body condition and may provide an indication of the nutrient and pollutant loads in an animal's habitat (e.g., Kennish 1997, Elumalai et al. 2005). The use of alternative N sources by estuarine consumers, and changes in their condition, potentially offer a means for the early detection of impacts in estuaries exposed to increased urbanisation.

The counteractive effects among the multitude of pollutants conveyed into estuaries from urbanisation add a degree of complexity in detecting any net biotic or ecological response to urbanisation. For example, the gradual seepage of groundwater-borne N from leaking sewer lines may lead to an increase in crab condition due to increased nutrient loads causing an increase in algal food availability (Kennish 1997) while occasional pulses of urban stormwater, containing heavy metals would impose an increased metabolic demand on crabs, resulting in a reduction in condition (Elumalai et al. 2005). However, exposure to these types of pollutants tends to vary through time and biotic responses to these pollutants are likely to reflect these temporal trends. Therefore, measurement of the temporal variance of biotic responses is likely to provide a more sensitive indication of urban impact (Underwood 1998).

Lake Coombabah is an estuarine lake located on the rapidly urbanising Gold Coast of southeast Queensland, Australia. The lake is considered an important fish habitat and resting site for migratory birds (DPIF 1994, Ramsar 2008). Extensive urbanisation has occurred within the lake's catchment over the last 50 years (Waltham 2002). Previous water monitoring data indicate that urban pollutants enter the lake at two influx sites, Coombabah Creek (CC), which drains the lake's main sub-catchment and a creek located next to a sewage treatment plant (STP) along the eastern shore of the lake (East Creek, EC). A stormwater pipe connecting the Helensvale (HV) residential estate with the western shore of the lake is another likely source of urban pollution to the lake. I investigated whether urban pollutants entering the lake at these three influx sites were having a localised effect on biota in the lake.

I measured the  $\delta^{15}\text{N}$  of the grey mangrove (*Avicennia marina*) and the tuxedo crab (*Australoplax tridentata*), microphytobenthos (MPB) biomass (as an estimate of benthic primary production), and crab condition (HI) around the influx sites. I tested the model that the influx of urban pollution would have localised impacts on nitrogen sources ( $\delta^{15}\text{N}$ ), MPB biomass (chlorophyll *a* concentration, Chl *a*), and crab condition (HI). These impacts would fluctuate between rainfall events and dry periods, due to an increased influx of urban runoff entering the lake following rainfall. I predicted that this fluctuation would increase the temporal variability of indicators nearest to the influx sites. Some variation of indicator values amongst the influx sites was also likely to occur due to natural differences between the sites. However, I predicted similar localised and temporal responses to urban influx at all three sites. I tested the following hypotheses:

(1) nutrient sources would be more affected by urban influx in places near (<10 m) influx sites than in places far (250 m) from influx sites. This would be demonstrated by enriched mean  $\delta^{15}\text{N}$  values in mangroves (*Avicennia marina*) and tuxedo crabs (*Australoplax tridentata*) near influx sites, as well as greater temporal variability and correlation with rainfall in mean crab  $\delta^{15}\text{N}$  values near influx sites and;

(2) MPB biomass and crab condition would be impacted more by urban pollution near influx sites (<10 m) than farther away (250 m). Depending on whether urban nutrients or toxic pollutants were predominant, mean Chl *a* and HI values would either increase or decrease in biota close to influx, as well as show greater temporal variability and correlation with rainfall at distances close to influx sites.

## 2.3 Methods

### 2.3.1 Study area and estimation of urbanisation within influx site sub-catchments

Lake Coombabah is a shallow, tide-dominated estuarine lake on the Gold Coast, southeast Queensland, Australia (Figure 2.1). The 465-ha lake has an average depth of ~1 m at high tide and is fringed by mangrove forests and saltmarsh (Lee et al. 2006). Extensive tidal mudflats contribute to the Lake's status as a Ramsar-listed migratory bird site. Lake Coombabah is gazetted as a Conservation Zone under the Moreton Bay Marine Park Zoning Plan (1997) and is a declared Fish Habitat Area under the Fisheries Act (1994). Extensive urban development has occurred within the lake's catchment over the last 50 years (Waltham et al. 2002).

Three influx sites draining urbanised sub-catchments flow into Lake Coombabah (Figure 2.1). The EC influx site drains a moderately urbanised sub-catchment which encompasses the Coombabah STP. The plant releases tertiary-treated wastewater through underground pipes to a discharge point at the Gold Coast Seaway, approximately 7 km away. There is no deliberate discharge of wastewater into EC. The CC site drains the lake's largest sub-catchment, comprising mainly urban and nature conservation land uses, with some mining activity in the upper catchment. The HV urban influx site drains the smallest of the three sub-catchments, and is predominantly urbanised (Table 2.1). This influx site is a stormwater pipe outlet, with ~ 20 metres of mangrove and saltmarsh riparian vegetation buffer from the lake margin. Traces of catchment runoff, such as terrestrial leaf litter and rubbish, across the saltmarsh indicate that stormwater flows through the riparian vegetation into the lake during heavy rainfall events. Characteristics of the influx sites indicate the presence of urban pollution (Table 2.1).

The extent of urbanisation surrounding each influx site was estimated using a census of the percentage of impervious cover (IC) within each of the influx sites' sub-catchments. This was estimated by overlaying the GIS layer for the lake's sub-catchment boundaries onto Google Earth satellite imagery recorded during 2004 (1 pixel  $\approx$  0.6 m<sup>2</sup>) (GE 2005, EPA 2006b). A grid overlay with points at 50 m intervals was applied to the imagery and the IC of each sub-catchment was determined from the proportion of grid points which fell on pavements, roads, roof tops, driveways, car parks, tennis courts and railroad tracks.

The sub-catchments surrounding the influx sites range in size between 77 and 2707 ha and all contain more than 20% IC (Table 2.1). Total nitrogen (TN) and faecal coliform

(FC) levels in excess of guideline values for 1994 – 2004 have previously been reported at the CC and EC influx sites (Table 2.1).

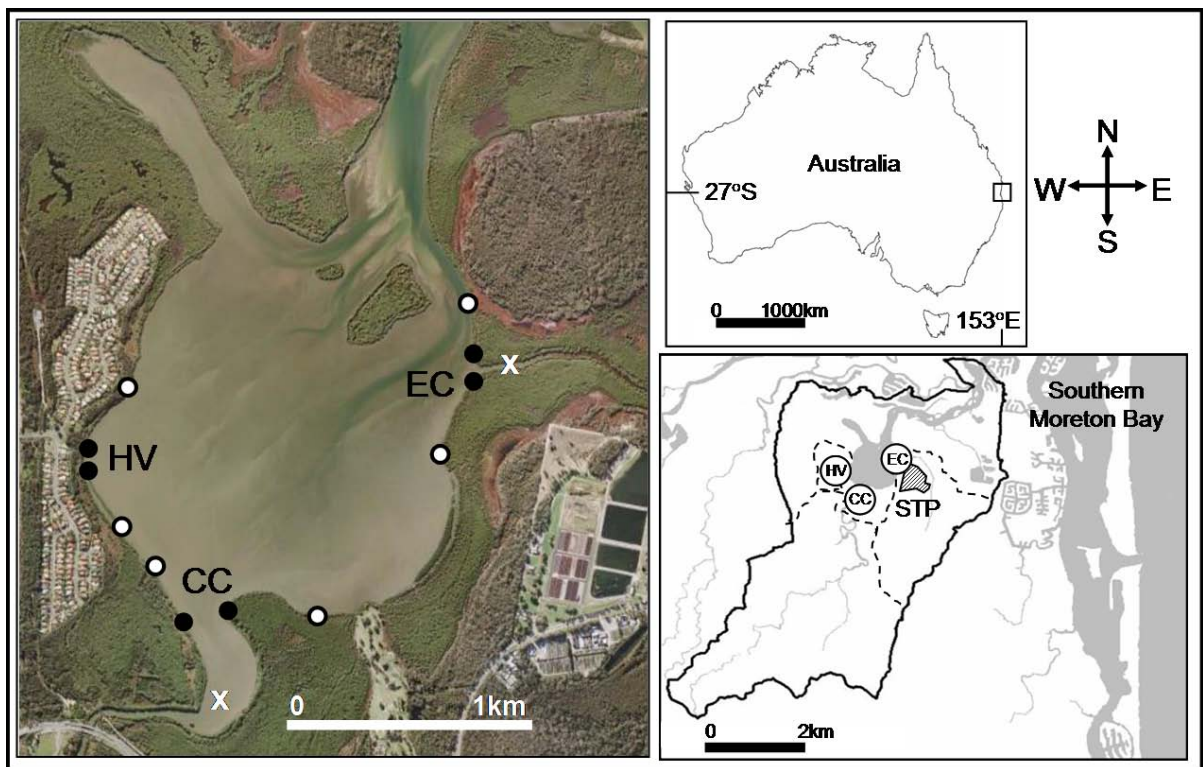


Figure 2.1. Map of Lake Coombabah, southeast Queensland, Australia, showing sub-catchment boundaries and the locations of the three influx sites East Creek (EC), Coombabah Creek, (CC), and the Helensvale stormwater drain (HV). The Coombabah STP is shown, adjacent to EC. X denotes where previous water quality samples were collected for measurement of total nitrogen (TN) and faecal coliform (FC) levels (Table 2.1)

Table 2.1. Characteristics of influx sites sampled within Lake Coombabah showing the percentage of catchment designated for each land use, (IC = impervious cover, CA = catchment area, TN = total nitrogen, FC = faecal coliforms, N/R = not recorded). TN and FC median values were measured at the sites indicated in Figure 2.1 each month during 1994 – 2004. Qld Water Quality (WQ) and Australian and New Zealand Environment and Conservation Council (ANZECC) guideline values for TN and FC are shown for comparison.

Influx site	Urban residential	Conservation & natural environments	Mining/waste	Production from natural environments	Water environments	Commercial	IC (%)	CA (ha)	TN ( $\mu\text{g L}^{-1}$ )	FC ( $\text{cfu100 L}^{-1}$ )
Coombabah Creek	34	40	4	9	9	4	21.5	2707	550	600
East Creek	28	47	7	0	18	0	20.8	838	635	600
Helensvale	93	5	0	0	2	0	40.6	77	N/R	N/R
Water quality guideline values									450	<150 (Primary contact)
Source			ABRS (2005)				This study	GCCC (2006)	GCCC (2004)	ANZECC & ARMCANZ (2000), GCCC (2004)

### 2.3.2 Sampling design and indicator collection

Samples ( $n = 10$ ) were collected haphazardly from the intertidal zone at distances near (<10 m) and far (250 m) from each urban influx site (Figure 2.1). The CC and HV influx sites are only separated by a distance of ~ 600 m. Therefore, in an effort to maintain independence between these two sites, indicators were not collected any farther than 250 m away from the influx sites. Collection occurred during daytime low tides over three consecutive days. Sampling was replicated six times in total, three times during the late dry season (12 Sep – 14 Oct 2005) and three times during the early wet season (7 Dec 2005 – 14 Jan 2006). At each sampling time the following samples were collected at both distances from the influx sites: 10 mature mangrove leaves (*Avicennia marina*), one each from 10 individual trees; 10 male crabs (*Australoplax tridentata*), standardised within a size range of 8–20 mm carapace width (CW). Female crabs were not used because egg production is likely to increase variation in the HI (Kyomo 1988); 10 cores of surface mud samples (1 cm deep, surface area of 5.7 cm<sup>2</sup>) for Chl *a* measurement, as a proxy for the biomass of MPB. Cores were wrapped in aluminium foil and put on ice immediately with the other indicator samples before freezing at -20°C in the laboratory for later analysis.

### 2.3.3 Rainfall

Rainfall data for the Gold Coast area during Sep – Oct 2005 and Nov 2005 – Jan 2006 were sourced from the Australian Bureau of Meteorology. Lag times were estimated for each of the indicators' likely responses to stormwater inputs and were applied to the rainfall data before testing for correlations with each of the indicators. These were based on the response times that similar indicators to those used in this study have had to pollutants and changes in nutrient sources, in previous studies (Table 2.2). A lag time of 3 – 6 days was applied to rainfall data that were tested for correlation with epipelagic Chl *a* (i.e. rainfall that occurred between three and six days prior to the date of sample collection); 6 – 9 days for crab hepatopancreas  $\delta^{15}\text{N}$  and crab HI and; 12 – 16 days for correlation with crab muscle  $\delta^{15}\text{N}$ .

### 2.3.4 Stable isotope analysis

Hepatopancreas and muscle samples were removed from the crabs under a dissecting microscope – taking care to remove any exoskeleton fragments from the tissues. The samples were then dried to constant weight at 60°C, homogenised, placed in tin capsules and

analysed for  $\delta^{15}\text{N}$  using a continuous flow GV Isoprime mass spectrometer.  $\delta^{15}\text{N}$  values were reported as:

$$\delta^{15}\text{N} (\text{‰}) = [(R_{\text{sample}} - R_{\text{standard}})/R_{\text{standard}}] \times 1000$$

Where  $R$  is the ratio  $^{15}\text{N}:^{14}\text{N}$ .  $\delta^{15}\text{N}$  values were reported relative to the primary standard, atmospheric air (Mariotti 1983). Prawn was used as a working standard for crab samples and flour as a working standard for the mangrove leaves. The standard deviation for 12 samples of each standard was lower than  $\pm 0.2\text{‰}$  for prawn and  $\pm 0.3\text{‰}$  for flour.

### 2.3.5 Crab HI

The hepatopancreas was carefully removed from each crab. After drying to a constant weight at  $60^{\circ}\text{C}$ , the hepatopancreas and remainder of the body tissues were weighed separately. HI was calculated as:

$$HI (\%) = \frac{H}{B} \times 100$$

Where  $H$  is the dry weight of the hepatopancreas and  $B$  is the dry weight of the remainder of the body. The HI was calculated using the hepatopancreas weight as a proportion of the body weight minus the hepatopancreas weight (Kennish 1997). This prevented any changes in the hepatopancreas from confounding the body weight, which would dampen any changes in the HI. This approach provides a more sensitive measure of changes in the crabs' condition than calculating the hepatopancreas as a proportion of the crabs' whole body weight.

### 2.3.6 MPB biomass (Chl *a*)

The biomass of MPB was estimated using the Chl *a* concentration of the surface mud samples. In the laboratory, the top 1 cm of mud was removed from each sediment core and placed into a centrifuge tube. Pigments were extracted using 90% acetone solution, neutralised with magnesium carbonate, shaken vigorously for 1 min and incubated overnight in the dark at  $3^{\circ}\text{C}$  (Lorenzen 1967, Yang et al. 2003). After incubation, the samples were centrifuged at  $4.4 \times 10^3$  rpm for 10 min and absorbance of the supernatant was measured at 665 and 750 nm using a spectrophotometer (Pharmacia Biotech Novaspec II) which was calibrated using a 90% acetone blank. In order to correct for any light absorbed by phaeopigments, one drop of 1 M HCl was added to each sample and the absorbance was measured again. Chl *a* concentrations were calculated using a modified version of the Lorenzen (1967) equation:

$$\text{Chl } a \text{ (}\mu\text{g m}^{-2}\text{)} = \frac{A \times K (665_i - 665_a) \times v}{\text{S.A.} \times l}$$

$A$  is the absorption coefficient of Chl  $a$  (11.0),  $K$  (2.43) equates the reduction in absorbance after acidification to the initial Chl  $a$  in each sample,  $665_i$  is the initial absorbance,  $665_a$  is the absorbance after acidification,  $v$  is the volume of acetone used for extraction (ml), S.A. is the surface area of mud sampled ( $\text{m}^2$ ),  $l$  is the path length of the cuvette (cm).

### 2.3.7 Data analyses

Hypotheses about the effects of distance from urban influx, influx site, sample time and season on mangrove leaf, crab muscle and hepatopancreas  $\delta^{15}\text{N}$ , crab HI and Chl  $a$ , were tested separately using four-factor ANOVAs. Distance (Di) was a fixed factor with two levels. Influx sites (In) were considered fixed with three levels. Season (Se) was considered to be fixed (dry and wet). Sample times (Ti) were random with six levels, three nested within each season. Hypotheses about correlations between rainfall and mean crab  $\delta^{15}\text{N}$ , HI and Chl  $a$ , measured at each sample time ( $n = 6$ ) were tested using Pearson's correlation. Due to the low number of replicate means, these correlations should be interpreted with caution. Temporal variability of the indicators was measured using the coefficient of variation (CV) of the grand mean of each mean indicator value between successive sample times within each season. This provided a standardised estimate of the variability between sample times relative to the mean (Quinn & Keogh 2002). A three-factor ANOVA was used to compare the CV for crab  $\delta^{15}\text{N}$ , HI and Chl  $a$  between distances (fixed, 2 levels) near and far from the sites, between influx sites (fixed, 3 levels) and between the dry and wet seasons of 2005 – 2006 (fixed, 2 levels). Cochran's tests were used to check data for homogeneity of variance, and where appropriate data were either natural log or square-root transformed prior to analysis. ANOVA interaction terms were pooled where  $p > 0.25$  and pooling resulted in a more powerful test (Underwood 1997).



Table 2.2. Previously observed response times of indicators to pollutants and changes in nutrient sources.

Indicator	Stressor/treatment	Response	Response time (days)	Source
MPB	Copper, Lead, Cadmium	Reduced growth rate	3	Moreno-Garrido et al. (2003)
Phytoplankton	Runoff containing N and sediments	Increased cell abundance and Chl <i>a</i>	3 – 6	Cox et al. (2006)
<i>Australoplax tridentata</i> (tuxedo crab)	Exposure to isotopically enriched carbon sources	$\delta^{13}\text{C}$ reflective of enriched carbon sources	7 – 14 (hepatopancreas) 14 – 28 (muscle)	Oakes (2006)
<i>Pomatoschistus minutus</i> (sand goby)	Diet switch	$\delta^{15}\text{N}$ reflective of new diet	3 (liver)	Guelinckx et al. (2007)
<i>Crassostrea gigas</i> (oyster)	Diet switch	$\delta^{15}\text{N}$ reflective of new diet	10 (whole tissue)	Dubois et al. (2007)
Juvenile <i>Portunus pelagicus</i> (blue swimmer crab)	Diet switch	$\delta^{15}\text{N}$ reflective of new diet	14 (muscle)	Young (2004)

## 2.4 Results

### 2.4.1 Localised mangrove and crab $\delta^{15}\text{N}$ responses to urban influx

Mangrove leaf  $\delta^{15}\text{N}$  values only differed between distances near and far from influx at the EC site with significantly enriched  $\delta^{15}\text{N}$  values near this site compared to farther away (Table 2.4, SNK,  $p < 0.01$ , Figure 2.2). Instead of showing a localised enrichment, crab muscle  $\delta^{15}\text{N}$  values were similar near and far from the influx sites during four of the six sampling times (Table 2.4, Figure 2.3). Following rainfall, muscle  $\delta^{15}\text{N}$  became significantly elevated in crabs near and far from influx sites during the second and fourth sampling times (SNK tests,  $p < 0.01$ , Figure 2.3). However, by the third and fifth sampling times, muscle  $\delta^{15}\text{N}$  had decreased significantly more in crabs farther from the influx sites than in crabs near the sites (SNK test,  $p < 0.01$ , Figure 2.3). Mean muscle  $\delta^{15}\text{N}$  values were positively correlated with rainfall in crabs near (Table 2.3) from the influx sites. Hepatopancreas  $\delta^{15}\text{N}$  also only differed between crabs near and far from the influx sites during the third and fifth sampling times (Time 3: Near:  $3.59 \pm 0.15\text{‰}$  vs. Far:  $3.30 \pm 0.12\text{‰}$ , and Time 5: Near:  $4.59 \pm 0.12\text{‰}$  vs. Far:  $4.21 \pm 0.13\text{‰}$ , Table 2.4, SNK test, both  $p < 0.05$ ). These responses showed no clear pattern with rainfall (Table 2.3). The temporal variability (coefficient of variation) of mean crab hepatopancreas and muscle  $\delta^{15}\text{N}$  showed no significant response to distance from the influx sites (Table 2.5).

### 2.4.2 Variation in mangrove and crab $\delta^{15}\text{N}$ between influx sites

Although not predicted, it is interesting to note that mangrove and crab  $\delta^{15}\text{N}$  values were most enriched at the EC site.  $\delta^{15}\text{N}$  was elevated in mangroves at the EC site (near and far) compared to in mangroves at the CC and HV sites throughout most of the sampling period (Table 2.4, SNK test, all  $p < 0.01$ ). Crab muscle  $\delta^{15}\text{N}$  values were also significantly elevated at EC compared to the other two sites throughout the entire sampling period (Table 2.4, SNK test,  $p < 0.01$ ; Figure 2.3). Hepatopancreas  $\delta^{15}\text{N}$  values displayed a similar pattern, but were only significantly more elevated at EC during the second, third and sixth sampling times (SNK test, all  $p < 0.05$ ). The comparison of indicator responses between influx sites also revealed that the significant correlation between crab muscle  $\delta^{15}\text{N}$  and rainfall only occurred at CC and HV (Pearson correlation:  $r = 0.91$  and  $r = 0.93$ , both  $p < 0.05$ ) but not at EC ( $r = 0.62$ ,  $p > 0.05$ , Figure 2.3).

The temporal variability (CV) of crab muscle  $\delta^{15}\text{N}$  displayed a significant interaction between the factors Influx site and Season (Table 2.5). Initially SNK tests found no significant pattern of interaction among the levels of these two factors. But pooling the non-significant three-way interaction term, Season  $\times$  Influx site  $\times$  Distance ( $p = 0.901$ ) provided enough power to detect a pattern among levels of Influx site and Season. Mean muscle  $\delta^{15}\text{N}$  showed greater temporal variability (CV) during the dry season than in the wet season at EC (SNK test,  $p < 0.01$ ). The opposite trend occurred at HV and CC with mean muscle  $\delta^{15}\text{N}$  displaying greater temporal variability in the wet season at both of these sites, although this was not significant (SNK tests,  $p > 0.05$ ).

### **2.4.3 MPB biomass (Chl *a*)**

Mean Chl *a* values for sediments collected far from the HV influx site had large variances (range: 3.6 to 181.9 mg m<sup>-2</sup>) compared to the mean Chl *a* values measured at the other sites. These data underwent various transformations, with a natural log transformation giving the best homogeneity of variance amongst the mean values of each factor. However, these data still breached the homogeneity of variance assumption; therefore these results should be interpreted with caution (Cochran's test,  $p < 0.05$ ). Chl *a* showed a significant interaction between Season, Influx site and Distance (Table 2.4) with some small differences between near and far from the influx sites, but no clear overall trend. Mean Chl *a* values showed no correlation with rainfall near or far from the influx sites (Table 2.3). Mean Chl *a* values showed no significant difference in temporal variability between distances from the influx sites, but did show significantly greater temporal variability during the dry season than in the wet season (Table 2.5).

### **2.4.4 Crab condition Indicator responses during the dry and wet seasons**

Crab HI responded interactively between Distance and Time(Season) (Table 2.4). HI was significantly lower in crabs near influx sites than in crabs far away during the first two sampling times (SNK test,  $p < 0.01$ ), but did not differ significantly between distances throughout the remaining times (SNK tests,  $p > 0.05$ ). During the third and fourth sampling times, HI appeared to decrease in crabs far from the sites and increase in crabs near the sites but these changes were not significant (SNK test,  $p > 0.05$ ). HI showed no correlation with rainfall in crabs at either distance from influx sites (all  $p > 0.05$ ). However, temporal variation in crab HI (CV) responded interactively between Distance and Influx site (Table 2.5). The CV of crab HI was significantly greater near the EC site than far from it (SNK test,

$p < 0.05$ , Figure 2.4), but did not differ between distances at CC or HV (SNK tests,  $p > 0.05$ ), although a similar trend occurred at the CC site (Figure 2.4).

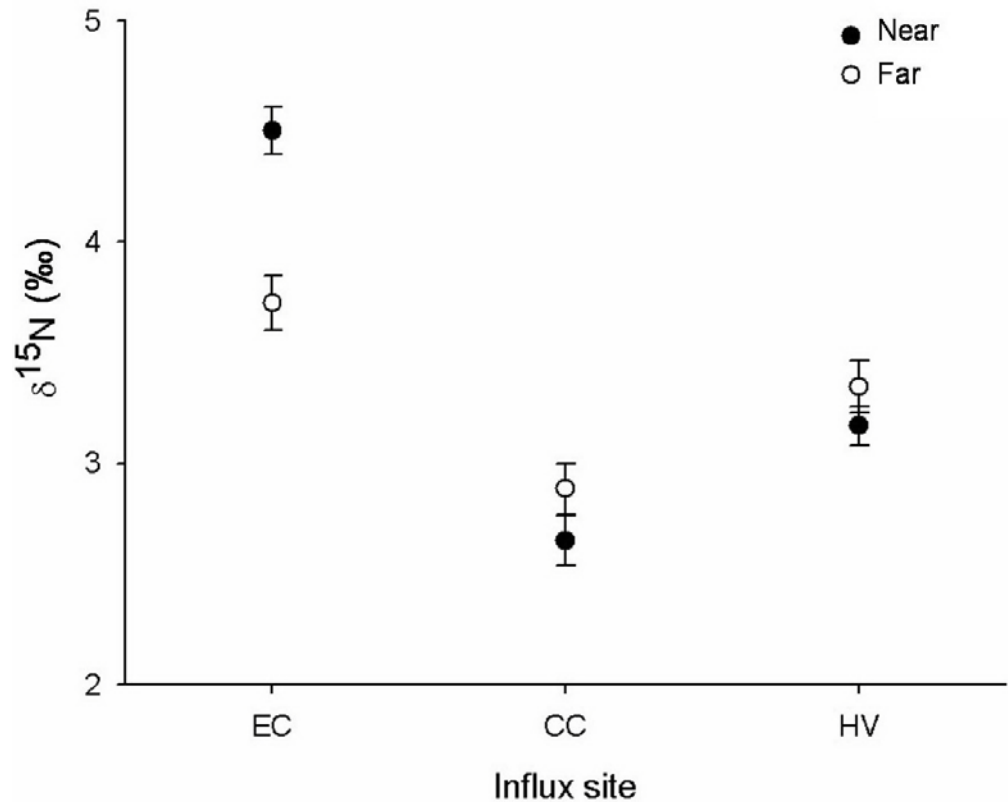


Figure 2.2. Mangrove  $\delta^{15}\text{N}$  (mean  $\pm$  SE), near ( $<10$  m), and far away from (250 m) the influx sites: East Creek (EC), Coombabah (CC), and Helensvale (HV).

Table 2.3. Coefficients for correlations between rainfall and mean indicator values measured during each sample time at distances near and far from the influx sites. Crab muscle  $\delta^{15}\text{N}$ , crab HI and Chl *a* data were natural log transformed to improve normality. Coefficients shown in bold denote significance at  $\alpha = 0.05$  (Pearson correlation).

Indicator	Near	Far
Crab $\delta^{15}\text{N}$ (Muscle)	<b>0.89</b>	<b>0.90</b>
Crab $\delta^{15}\text{N}$ (Hepatopancreas)	0.61	0.62
MPB biomass (Chl <i>a</i> )	-0.20	-0.28
Crab condition (HI)	0.33	-0.64

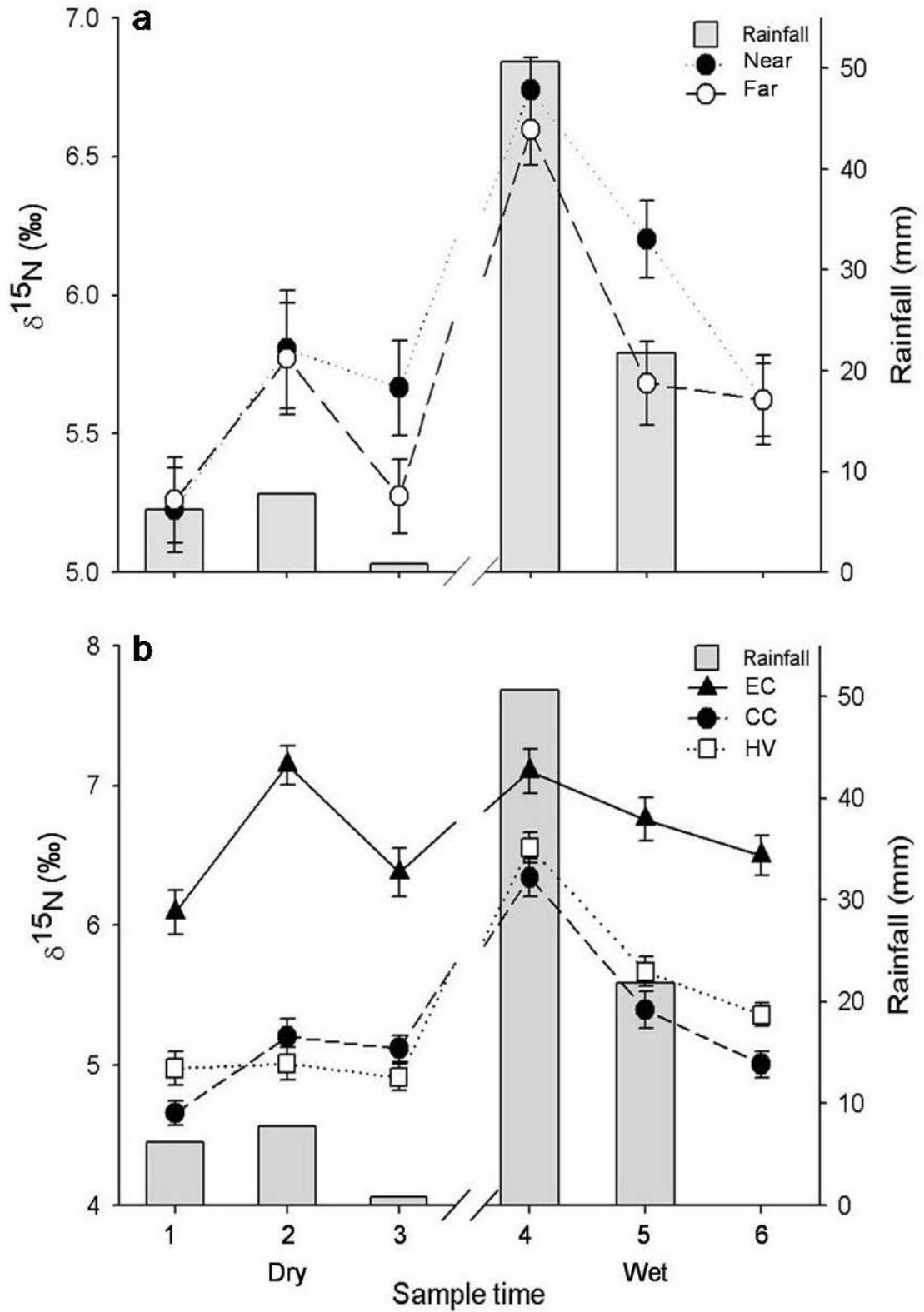


Figure 2.3.  $\delta^{15}\text{N}$  values (mean + SE) of a) muscle tissue in crabs sampled near and far from influx sites and b) muscle tissue in crabs at the three influx sites: East Creek (EC), Coombabah Creek (CC), and Helensvale (HV) with total rainfall recorded 12 – 16 days prior to sampling. Crabs were sampled six times over the dry and wet seasons of 2005 – 2006.

Table 2.4. ANOVA examining the effects of Season (Se), Time nested in Season (Ti(Se)), Influx site (In) and Distance from urban influx (Di) for each indicator. Crab muscle  $\delta^{15}\text{N}$ , crab HI and Chl *a* data were natural-log transformed. The homogeneity of variance assumption was satisfied for all indicators (Cochran's Test,  $p > 0.05$ ) except Chl *a*.  $p$  values shown in bold denote significance at  $\alpha = 0.05$ .

Source	df	Mangrove		Crab hepatopancreas		Crab muscle		Crab Hepatosomatic Index (HI)		Chl <i>a</i>	
		<i>F</i>	<i>p</i>	<i>F</i>	<i>p</i>	<i>F</i>	<i>p</i>	<i>F</i>	<i>p</i>	<i>F</i>	<i>p</i>
Se	1	0.04	0.851	0.74	0.438	3.09	0.154	1.43	0.299	0.46	0.531
Ti(Se)	4	8.41	<b>&lt;0.001</b>	88.29	<b>&lt;0.001</b>	34.75	<b>&lt;0.001</b>	0.58	0.674	29.60	<b>&lt;0.001</b>
In	2	36.19	<b>&lt;0.001</b>	15.31	<b>0.002</b>	53.99	<b>&lt;0.001</b>	1.72	0.240	63.24	<b>&lt;0.001</b>
Di	1	2.93	0.162	0.91	0.395	3.08	0.154	4.37	0.105	5.56	0.078
Se $\times$ In	2	0.02	0.976	1.29	0.327	2.89	0.113	1.27	0.332	5.35	<b>0.034</b>
Se $\times$ Di	1	0.42	0.553	0.27	0.632	0.09	0.785	2.64	0.180	0.63	0.473
In $\times$ Ti(Se)	8	2.32	<b>0.020</b>	10.69	<b>&lt;0.001</b>	4.21	<b>&lt;0.001</b>	0.93	0.494	1.10	0.361
Di $\times$ Ti(Se)	4	0.70	0.591	2.90	<b>0.022</b>	3.27	<b>0.012</b>	2.48	<b>0.044</b>	2.24	0.064
In $\times$ Di	2	8.30	<b>0.011</b>	1.39	0.303	3.94	0.064	1.68	0.246	3.90	0.066
Se $\times$ In $\times$ Di	2	1.04	0.396	0.05	0.948	1.51	0.277	1.13	0.371	5.28	<b>0.035</b>
Di $\times$ In $\times$ Ti(Se)	8	1.78	0.081	1.38	0.204	1.50	0.156	1.83	0.070	0.67	0.720
Residual	324										

Table 2.5. ANOVA test of variability (CV) among Seasons (Se), Influx site (In) and Distance (Di) from influx on all indicators. Chl *a* data were square-root transformed before analysis. The assumption of homogeneity of variance was satisfied for all indicators (Cochran's Test,  $p > 0.05$ ).  $p$  values shown in bold denote significance at  $\alpha = 0.05$ .

Source	d.f	Coefficient of Variation (CV) of indicators							
		Crab condition (HI)		Hepatopancreas $\delta^{15}\text{N}$		Muscle $\delta^{15}\text{N}$		Chl <i>a</i>	
		<i>F</i>	<i>p</i>	<i>F</i>	<i>p</i>	<i>F</i>	<i>p</i>	<i>F</i>	<i>p</i>
Se	1	0.21	0.658	0.27	0.614	0.43	0.521	11.29	<b>0.006</b>
In	2	0.74	0.498	0.31	0.737	0.71	0.509	0.06	0.942
Di	1	2.88	0.116	0.07	0.793	0.04	0.854	0.31	0.587
Se $\times$ In	2	0.19	0.829	1.41	0.282	5.45	<b>0.018</b>	0.21	0.812
Se $\times$ Di	1	0.2	0.662	0.31	0.585	0.08	0.776	2.89	0.115
In $\times$ Di	2	4.39	<b>0.037</b>	0.1	0.906	0.43	0.658	1.06	0.375
Se $\times$ In $\times$ Di	2	0.11	0.894	0.03	0.973			0.54	0.595
Residual	12								

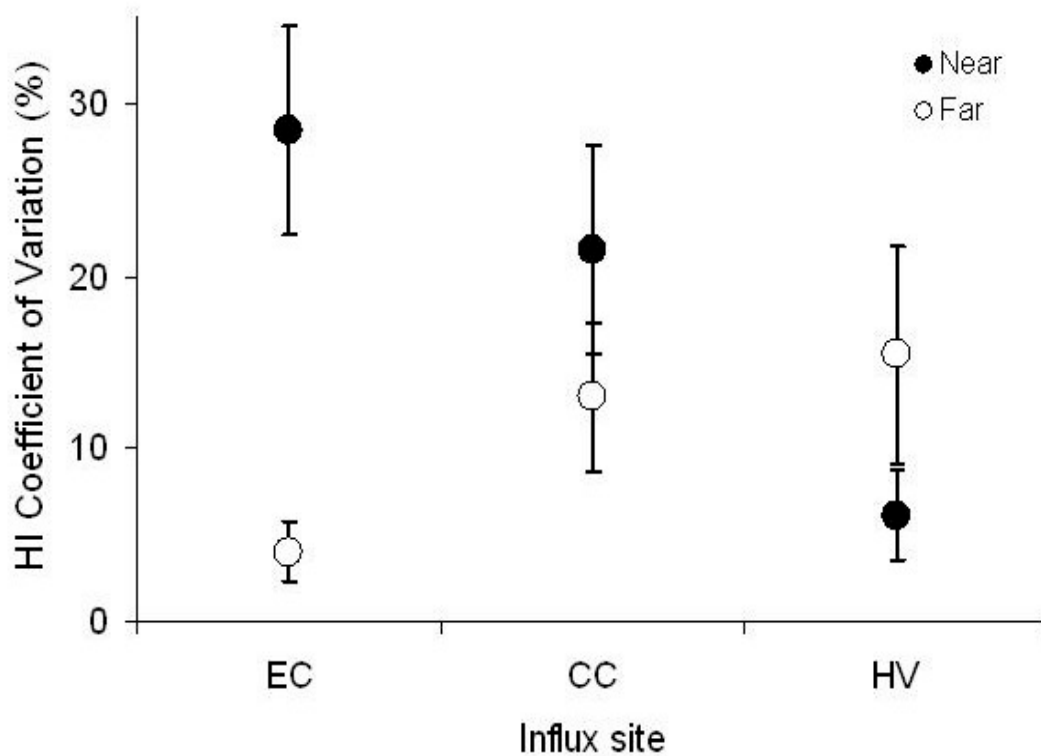


Figure 2.4. Coefficient of variation (CV, mean  $\pm$  SE) of mean crab HI near and far from the three influx sites. Crabs were sampled on three occasions during the wet and dry season of 2005 – 2006 and the mean CV of the grand mean of HI between successive sample times was measured,  $n = 4$  at each distance from each influx site.

Table 2.6. Summary of indicator responses in relation to the main hypotheses tested.

Indicator	Mean	Temporal variability	Correlation with rainfall
Mangrove $\delta^{15}\text{N}$	Mean $\delta^{15}\text{N}$ was more enriched near urban influx than farther away at the EC site, which is adjacent to a STP. Distance $\times$ Influx site.	Not tested	Not tested
Crab $\delta^{15}\text{N}$ (Muscle)	Mean $\delta^{15}\text{N}$ was more enriched near urban influx sites than farther away during the 3 <sup>rd</sup> and 5 <sup>th</sup> sampling times. Distance $\times$ Time (Season).	No difference between near and far from the influx sites.	Positive correlation between mean $\delta^{15}\text{N}$ and rainfall – however this was no greater near the influx sites than farther way.
Crab $\delta^{15}\text{N}$ (Hepatopancreas)	Mean $\delta^{15}\text{N}$ was more enriched near urban influx than farther away during the 3 <sup>rd</sup> and 5 <sup>th</sup> sampling times. Distance $\times$ Time (Season).	No difference between near and far from the influx sites.	No correlation with rainfall.
MPB biomass (Chl <i>a</i> )	No consistent pattern of response to distance from the urban influx sites. Season $\times$ Influx site $\times$ Distance	No difference between near and far from the influx sites.	No correlation with rainfall.
Crab condition (HI)	Mean crab HI was depleted closer to the urban influx sites during the 1 <sup>st</sup> and 2 <sup>nd</sup> sampling times. Distance $\times$ Time (season).	Mean crab HI was more temporally variable near the EC influx site. Influx site $\times$ distance.	No correlation with rainfall.



## 2.5 Discussion

### 2.5.1 Mangrove and crab $\delta^{15}\text{N}$ indicate an influx of sewage N and N in urban runoff

Approximately 90% of the variation in crab muscle  $\delta^{15}\text{N}$  was explained by the amount of rainfall 12 – 16 days prior to each sampling time. Following rainfall the enrichment in crab  $\delta^{15}\text{N}$  penetrated at least 250 metres into the Lake from the influx sites, indicating that stormwater runoff delivers a substantial pulse of urban N, which is assimilated into the Lake's food web (Dillon & Chanton 2008). Increased groundwater flows following rainfall are also likely to convey isotopically enriched N from leaky sewer lines into the creeks flowing to the Lake (Valiela et al. 1997). The slower recovery of muscle  $\delta^{15}\text{N}$  to pre-rainfall values in crabs near influx sites, compared to  $\delta^{15}\text{N}$  in crabs far from the sites, indicates that urban N has a longer residence time closer to the influx sites.

A comparison of the three sites revealed that the positive correlation between rainfall and crab muscle  $\delta^{15}\text{N}$  was only significant at CC and HV, indicating that urban runoff and increased groundwater flows are the main sources of urban N at these two sites (Valiela et al. 1997, Steffy & Kilham 2004, Dillon & Chanton 2008). Recycled wastewater is used for irrigation and the construction of artificial water features in local parks. Following rainfall, increased stormwater and groundwater flows may also convey this source of enriched  $\delta^{15}\text{N}$  into these two sites. The lack of correlation between crab muscle  $\delta^{15}\text{N}$  and rainfall at EC, together with the greater temporal variation of crab muscle  $\delta^{15}\text{N}$  during the dry season, indicate that factors other than rainfall contribute to an influx of urban N at this site. Despite the temporal fluctuations in crab muscle  $\delta^{15}\text{N}$  at EC,  $\delta^{15}\text{N}$  values were consistently greater at this site than at CC and HV, indicating that a constant or frequent influx of urban N enters the Lake at EC. Mangrove leaf  $\delta^{15}\text{N}$  values displayed a localised enrichment at EC and were also more enriched at EC than at CC and HV throughout most of the sampling period. The N contained in mature mangrove leaves is considered to be reflective of long-term (~ 1 year) sediment nutrient patterns (Boto & Wellington 1983, Alongi et al. 2005, Lin et al. 2007). The enriched  $\delta^{15}\text{N}$  value of mangroves at EC may also be indicative of either a constant or frequent influx of urban N at this site.

The Coombabah STP is located within the EC sub-catchment. There is no deliberate discharge of tertiary-treated wastewater into Lake Coombabah from the plant, but it is in close proximity to EC (< 500 m). It is possible for N in urban wastewater to leak from any cracks in the STP's infrastructure, into surrounding groundwater and into EC, or to overflow into EC. This connectivity may be further enhanced following periods of high rainfall, or spring tides.

The  $\delta^{15}\text{N}$  of estuarine producers and consumers has been positively correlated with the presence of tertiary-treated wastewater in a number of studies. For example, Savage (2005) reported that macroalgae (*Fucus vesiculosus*)  $\delta^{15}\text{N}$  increased to values > 7‰ within 10 km of a tertiary-treated sewage outfall in Himmerfjorden Bay, Sweden. McClelland et al. (1997) also showed an enrichment in the  $\delta^{15}\text{N}$  values of estuarine invertebrates in estuaries receiving greater wastewater N loads. In Moreton Bay, Australia, elevated  $\delta^{15}\text{N}$  (> 5‰) values have been observed in *Avicennia marina* up to 5 km away from tertiary-treated sewage outfalls (Costanzo et al. 2001). The elevated  $\delta^{15}\text{N}$  values observed in crabs and mangroves at EC strongly suggest that urban N from the adjacent STP enters Lake Coombabah at this site.

Alternatively, the potential occurrence of an isotopic riverine to marine gradient across Lake Coombabah may explain the elevation in mangrove and crab muscle  $\delta^{15}\text{N}$  at EC. Marine sources of N are more enriched in  $^{15}\text{N}$  than riverine sources (Wada et al. 1975, Michener & Schell 1994). Of the three sites, EC is located closest to the seaward entrance of the Lake. However, conductivity levels measured at the EC site ( $32.86 \pm 1.64 \text{ mS cm}^{-1}$ ) between 1992 and 2004 were similar to those measured at the CC site ( $32.46 \pm 1.75 \text{ mS cm}^{-1}$ ), indicating a similar level of marine influence at all of the influx sites around the lake (GCCC 2004). Furthermore, if the enrichment in  $^{15}\text{N}$  at EC was due to a marine influence, one would expect to see an enrichment of  $\delta^{15}\text{N}$  in mangroves farther away from the creek. Instead, they were most elevated near the creek, indicating that the enriched  $\delta^{15}\text{N}$  values at this site were due to an influx of urban N from the sub-catchment, rather than from marine sources.

Crab hepatopancreas  $\delta^{15}\text{N}$  responded interactively to the same factors as crab muscle  $\delta^{15}\text{N}$ , but was more variable and showed no correlation with rainfall. Due to the wide array of metabolic processes (e.g. nutrient storage, metabolism of energy reserves) carried out in the hepatopancreas, isotopic turnover rates are likely to vary more than in muscle tissues. Unfortunately, studies examining tissue-specific rates of change in  $\delta^{15}\text{N}$

following shifts in nutrient sources are scarce, particularly for crustaceans. Recent studies on fish have shown that the isotopic turnover rates in liver are highly variable and reduce the statistical power to detect subtle shifts in nutrient sources, whereas, the white muscle tissue of fish is less variable and considered a more reliable tissue for detecting isotopic shifts in nutrient sources (Pinnegar & Polunin 1999, Sweeting et al. 2005). The greater variability observed in the  $\delta^{15}\text{N}$  of crab hepatopancreas in this study also suggests that this tissue is less reliable for tracing dietary shifts in crabs – future studies may benefit more by focusing on isotopic responses in the less variable muscle tissues of crabs.

### **2.5.2 MPB biomass and crab condition responses to urban influx**

Crab HI showed greater a greater degree of temporal variation near EC than farther away indicating a localised effect on crab condition, potentially linked with a fluctuating influx of N from the STP and urban runoff. Crab HI varied to a similar extent through time in crabs at CC, although this response was not as localised as it was at EC. The CC site drains most of the lake's upper catchment and a greater volume of catchment runoff is likely to enter this site. This may contribute to a greater dispersal of urban influx and its effects on crab HI at CC. Crab condition appeared to fluctuate least near the HV site, indicating that crabs near this site are exposed to less urban influx, than crabs at EC and CC. Although the HV site has the most urbanised sub-catchment of the three sites, it is likely to receive smaller, less frequent flows of urban influx than CC and EC. The HV site is adjacent to a stormwater drain, with a ~20 metres of saltmarsh and mangrove vegetation, separating the site and the drain. Because the stormwater drain does not directly connect to the HV site, urban runoff during small rainfall events is likely to be intercepted and infiltrated by the vegetation before it reaches the site (Birch et al. 2004, Walsh et al. 2005). Therefore, an influx of urban pollution is only likely to occur at this site following large rainfall events. This was demonstrated by the lack of elevation in crab  $\delta^{15}\text{N}$  values at HV during the second sampling time following a small rainfall event (~8 mm) and the notable elevation in crab  $\delta^{15}\text{N}$  values at HV during the fourth sampling time, following a notably larger rainfall event (~50 mm).

The increased temporal variability in crab condition at EC and CC is indicative of temporal shifts in the types of urban pollutants entering these sites. For example, crab condition may increase due to increased food availability at times when urban N sources are the main pollutants flowing into EC and CC (e.g., Kennish 1997). When pollutants associated with urban stormwater, such as sediments and metals, flow into these sites,

crab condition may become depleted due to metabolic stress (Connell et al. 1999, Elumalai et al. 2005). This increased temporal variability in crab condition has important implications for the health of crabs around these influx sites. During times of reduced condition, crab populations are likely to be less resilient to additional disturbances (e.g. major flood events or exposure to acid sulphate soils), making them more vulnerable to local extinction (Underwood 1998). The burrowing activities of crabs such as *Australoplax tridentata* contribute greatly to the structure and function of mangrove ecosystems. Burrowing activities can increase the micro-topography of the forest floor, reduce algal cover, increase the flow of aerated seawater through mangrove sediments and increase the exchange of organic carbon across the sediment surface (Warren & Underwood 1986, Taylor & Allanson 1993, Ridd 1996). The crabs themselves are an important trophic link between benthic primary producers and fish in estuarine food webs (Sheaves & Moloney 2000). Some species of ocypodid crab (*Macrophthalmus* ad *Uca* spp.) are also an important source of prey to migratory birds (Bildstein et al. 1989, Dann 1999, Zharikov & Skilleter 2004). Therefore, the impact that urban influx is having on crab condition also has important implications for the ecosystem health of Lake Coombabah.

Mean crab body condition was too variable to show any clear pattern in response to urban influx. Many of the complex physiological processes in the crab hepatopancreas, including nutrient absorptive processes and metabolic mechanisms are under-investigated (Verri et al. 2001). Variability in the rates and direction at which these processes occur in response to different urban stressors is likely to have prevented the detection of an overall mean response to urban influx. MPB biomass showed no clear response to urban influx. Although, the localised depletion of Chl *a* near EC during the wet season may have been caused by an increased influx of urban pollutants, driven by larger rainfall events and; decreased Chl *a* in sediments far from CC during the wet season may indicate a greater dispersal of urban influx at this site.

### **2.5.3 Comments on experimental design**

The mangrove, crab  $\delta^{15}\text{N}$  and crab condition responses, indicated a localised impact at the EC site, but urban pollutants also enter the lake through the CC and HV influx sites following rainfall. However, due to a lack of pristine (control) influx sites, it was not possible to account for any of the effects that natural stressors, such as freshwater inputs, may have also had on biota. For example, the increased temporal variability of

crab condition near the EC site may have been due to a combination of urban influx and natural stressors. A comparison of biotic responses between pristine and urbanised sites would enable separation of these effects. Alternatively, an experiment testing for any relationship between the responses of biota and the extent of urbanisation surrounding different estuaries would enable a better assessment of estuarine responses to urban pollutants, in the presence of natural stressors. Finally, although Lake Coombabah is a typical sub-tropical estuarine lake, it was the only system surveyed in this experiment. While these results are considered to be representative indicators for the biotic condition of Lake Coombabah, any interpretation of them in relation to other estuarine lakes should recognise this limitation.

#### **2.5.4 Conclusions**

This study tested whether urban pollutants were having a localised impact on estuarine biota around three urban influx sites within Lake Coombabah. A localised enrichment of  $\delta^{15}\text{N}$  occurred in biota at one of the three sites, EC, indicating that urban sources of N enter the lake through this site from the adjacent STP. An increase in the temporal variability of crab condition at this site indicates that the assimilation of urban N sources and exposure to other urban pollutants is having a localised impact on biota at this site. Responses to urban pollutants were not as localised at the two remaining influx sites (CC and HV).  $\delta^{15}\text{N}$  values in crabs and mangroves were similar at distances near and far from these sites, but became enriched at both distances following rainfall. Positive correlations between rainfall and crab muscle  $\delta^{15}\text{N}$  around these sites indicate that isotopically enriched urban N enters the lake through these sites in urban runoff. The only localised response displayed at these two sites was a faster recovery of  $\delta^{15}\text{N}$  to pre-rainfall values in crabs located farther away from the sites. Collectively, these responses indicate that urban runoff and the STP convey nutrient sources into Lake Coombabah. This is having a localised effect on biota at the EC influx site which drains a moderately urbanised catchment and potentially has some degree of connectivity with the adjacent STP. These effects should be considered as an early warning signal of degradation, and effort should be made to mitigate the influx of pollution entering this increasingly urbanised estuary.

## Chapter 3. Responses of mangrove biota in estuarine lakes to increased urbanisation around southeast Queensland

### 3.1 Abstract

Urbanisation in coastal catchments around southeast Queensland is booming and imposing increasing pressure on estuaries in the region. In order to manage this urban growth sustainably, we need to understand how estuarine ecosystems are responding to increasing urbanisation pressures. This study investigates the responses of mangrove biota to increasing urbanisation.  $\delta^{15}\text{N}$  values of the grey mangrove (*Avicennia marina*), red-fingered marsh crab (*Parasesarma erythrodactyla*), and tuxedo crab (*Australoplax tridentata*), together with the biomass of microphytobenthos, MPB, (Chlorophyll *a* concentration, Chl *a*) and crab condition (hepatosomatic index, HI) were measured in five estuarine lakes each differing in the extent of urbanisation within their catchments, which was measured as the percentage of impervious cover (IC) within each catchment.

Mangrove and crab  $\delta^{15}\text{N}$  values indicated that urban nutrients enter the lakes' food webs from adjacent sewage treatment plants (STPs) and septic tank systems, regardless of the amount of urbanisation within their catchments. MPB biomass and crab condition were combined to form a composite indicator of biotic condition (BCI), which displayed a steep decline with increased urbanisation when IC was adjusted for the lakes' sensitivity to catchment disturbance (i.e. the ratio of catchment area to lake area). This decline in biotic condition showed no relationship with the presence of urban nutrients in the lakes, indicating that urban pollutants that inhibit primary production and consumer condition have a greater impact on the biotic condition of the lakes than urban nutrients.

This study supports the model that urbanisation is having a growing impact on the biota of receiving estuarine lakes. This appears to be related to greater IC in more urbanised catchments, which is likely to increase the runoff of urban pollutants into the lakes. The need to account for attributes which are likely to influence the sensitivity of estuaries to catchment disturbance were highlighted by this relationship. Although catchment imperviousness was the main influence contributing to the decrease in biotic condition, sewage infrastructure appeared to be the predominant factor contributing to the presence of urban N sources in the lakes.

### 3.2 Introduction

The current population boom of southeast Queensland is imposing a number of stressors on the region's coastal environments. During the next 20 years, the human population around this region is projected to increase from 2.9 to 4 million (OUM 2004). As urban developments are built to accommodate the growing population, considerable areas of permeable forested and rural lands are expected to be converted into impervious areas, such as buildings, roads, and car parks. Increases in these impervious areas impact the ecology of receiving waterways by reducing the infiltration of rain water into naturally pervious soils and channelling greater urban runoff loads into receiving waterways following rainfall (Arnold & Gibbons 1996, CWP 2003). This urban runoff delivers diffuse pollutants such as metals, petroleum hydrocarbons and sediments into urbanised waterways (Sanger et al. 1999a, b, Drapper et al. 2000, Bay et al. 2003). In southeast Queensland, diffuse sources of nutrient and sediment loads entering waterways are expected to increase by more than 50% over the next 20 years (EPA 2007).

Urbanisation also increases the flow of pollutants from point sources, such as sewage treatment plants (STPs), into receiving estuaries (Downing & Peterka 1978, McClelland & Valiela 1998). The release of nutrients from such point sources into estuaries around southeast Queensland is also expected to increase by 50% by 2026 (EPA 2007). Future plans to recycle wastewater for potable purposes are expected to reduce the current volumes of tertiary-treated wastewater released into the regions estuaries (Southeast Queensland Water Strategy – draft 2008). However, there remains some uncertainty over the release of the reverse osmosis concentrates of nutrients and other pollutants removed from the wastewater during purification processes. Long-term monitoring data by the Queensland Environmental Protection Agency identify sediment and nutrient inputs as the primary pollutants of concern in urbanised estuaries around the region (EPA 2007). Responsible management of the pollutants from diffuse and point sources, together with careful monitoring of estuarine responses to growing urbanisation pressures, are vital in preventing the regions estuaries from becoming degraded.

The percentage of catchment area covered by impervious surfaces (Impervious Cover, IC) has been positively correlated with housing density and urban runoff volumes (Schueler 1987, Arnold & Gibbons 1996). Previous studies have also shown a relationship between IC and the water quality and biotic responses of receiving waterways with notable adverse effects occurring in water quality when IC exceeds 10 – 20% and in biota when IC exceeds 20 – 30% (e.g., Arnold & Gibbons 1996, Mallin et al.

2000, Walsh et al. 2001, CWP 2003, Holland et al. 2004). This relationship potentially makes IC a convenient predictor of urban impacts in receiving estuaries, and a useful tool for urban managers in identifying threshold values for urbanisation that can be applied to future urban plans.

Most studies of biotic responses to IC however, have been made in freshwater stream systems (e.g., Arnold & Gibbons 1996, Walsh et al. 2001, CWP 2003), with relatively few studies assessing the relationship in estuarine systems (e.g., Sanger et al. 1999a, Holland et al. 2004). These studies were done in tidal creek estuaries in South Carolina, USA, which had macro-tidal ranges of 4.6 – 7.9 m (Van Dolah et al. 2008). Tidal flushing appears to play an important role in mitigating anthropogenic inputs in these systems, reducing the relevance of these results to less well flushed estuaries with smaller tidal ranges (Dame et al. 2000). Further investigation of the relationship between IC and biotic responses in a broader range of estuarine systems will contribute to understanding the generality of this relationship in estuaries.

In Chapter 2 the  $\delta^{15}\text{N}$  values of mangrove biota indicated that groundwater seepage from an adjacent STP and urban runoff contributed to an influx of urban nutrients into Lake Coombabah, thus altering the nutrient sources of the estuary. An increase in the temporal variability of crab condition near two creeks, one draining the lakes urbanised upper catchment and the other draining a sub-catchment encompassing the Coombabah STP, indicated that exposure to urban nutrients and other urban pollutants is starting to impact the health of biota in the lake. Chapter 2 provided a useful indication of the sources of urban pollution within Lake Coombabah and of the scale of biotic responses to these pollutants. However, it did not enable an assessment of how estuarine biota respond to increasing urbanisation, a very relevant relationship to understand considering the present rate of urban growth along the southeast Queensland coast. An assessment of biotic responses in estuarine lakes surrounded by different degrees of urbanisation would enable an estimate of any relationship between urbanisation and the condition of estuarine biota.

Preliminary data collected from two estuarine lakes, one moderately urbanised (Lake Coombabah, IC: 24%) and the other slightly urbanised (Lake Weyba, IC: 10%) indicated that urbanisation contributed to a greater assimilation of urban nitrogen (N) sources by biota with significant enrichment of mean crab  $\delta^{15}\text{N}$  values in the moderately urbanised lake (*Parasesarma erythrodactyla* muscle  $\delta^{15}\text{N}$ :  $7.0 \pm 0.5\text{‰}$ ) compared to in



the slightly urbanised lake ( $\delta^{15}\text{N}$ :  $4.2 \pm 0.4$ ). Crabs were also in poorer condition in the more urbanised of the two lakes (mean HI:  $2.9 \pm 0.8\%$  vs.  $6.1 \pm 1.2\%$ ) indicating that urban pollutants impose a greater metabolic demand on crabs. These differences provided evidence to suggest that biota collected from estuarine lakes surrounded by increasing levels of urbanisation were likely to show a gradient of response to urbanisation.

In this chapter I investigated whether a relationship exists between the nutrient sources and condition of mangrove biota and the level of urbanisation surrounding five estuarine lakes around southeast Queensland. Mangrove biota were collected from the lakes on six occasions over a one year period. This enabled a time integrated assessment of biotic condition, as well as a snap shot of the extent of temporal variability in the lakes. The IC within each lake catchment was used as a measure of urbanisation. However, due to differences in the size of the lakes and their catchments, sensitivity to urbanisation was likely to differ between the lakes (Horton & Eichbaum 1991). Therefore, an adjusted version of IC (AIC) was also used as a measure of urbanisation so that differences in lake sensitivity could be taken into account.

Based on the biotic responses observed in the preliminary data, I tested the model that with increasing catchment urbanisation, mangrove biota would show a greater assimilation of urban nutrient sources and that benthic primary production and consumer condition would decrease. The  $\delta^{15}\text{N}$  of mangrove biota was applied as a tracer for urban N sources, MPB (microphytobenthos) biomass (Chl *a*), as an estimate of benthic primary production, and the hepatosomatic index (HI) of two species of mangrove crab as an indicator of consumer condition. Urbanisation was also likely to increase the temporal variability of these indicators, due to increased IC altering the hydrology of the lakes' catchments, channelling greater concentrations of urban runoff into the more urbanised lakes.

The combination of biotic indicators into composite indices can offer a better estimate of the ecosystem level response to disturbance, particularly when the indicators are selected from multiple trophic levels or niches in the ecosystem. It also improves the ease of communicating ecological responses to environmental managers (Galloway et al. 2004, Bilkovic & Roggero 2008). Indicators that showed clear responses to urbanisation were also combined to provide a composite index of biotic condition (biotic condition index, BCI).

I tested the following hypotheses:

(1) the assimilation of urban N by the grey mangrove (*Avicennia marina*), ocypodid crab (*Australoplax tridentata*, tuxedo crab), and grapsid crab (*Parasesarma erythrodactyla*, red-fingered crab), would increase with urbanisation. This would be demonstrated by an enrichment in mangrove and crab  $\delta^{15}\text{N}$  values with increased IC and/or AIC;

(2) The biomass of MPB, measured as Chl *a*, and crab condition (HI), would decrease with greater IC and/or AIC;

(3) A composite index of biotic condition formed using the indicators in this study would also show a decrease with greater IC and/or AIC and;

(4)  $\delta^{15}\text{N}$ , crab HI, and, Chl *a* would show stronger correlation with rainfall and larger temporal variation in lakes surrounded by greater IC and/or AIC.

### 3.3 Methods

#### 3.3.1 Study area and estimation of urbanisation

Five estuarine lakes located around southeast Queensland, Australia, were selected as study sites (Figure 3.1). Lakes Cooroibah, Weyba, Coombabah, Cobaki, and Terranora are sections of subtropical estuaries with semidiurnal tidal cycles (< 1.5 m range) and similar habitat types, comprising mainly mangroves (*Avicennia marina* and *Rhizophora stylosa*) and saltmarsh couch (*Sporobolus virginicus*). The lakes vary in size and in extent of surrounding urbanisation. A summary of the catchment land uses and lake characteristics is outlined in Table 3.1. The extent of urbanisation surrounding the lakes was estimated as the IC within each lake's catchment. This was measured using the methods described in Chapter 2.

According to IC estimates, Lake Cooroibah was the least urbanised of the lakes, with only 0.3% IC. Lake Cobaki was the second least urbanised, followed by Lakes Weyba and Terranora, with Lake Coombabah being the most urbanised with 23.8% IC (Table 3.1). Due to differences in catchment area and lake size, sensitivity to urbanisation was likely to differ between the lakes (Horton & Eichbaum 1991). In order to adjust for these differences, IC was multiplied by the ratio of the catchment area to lake area for each lake as shown below:

$$\text{Catchment area (ha) : Lake area (ha)} \times \text{IC (\%)}$$

Previous studies have used the ratio of catchment area to estuary volume as a measure of sensitivity (Horton & Eichbaum 1991). Unfortunately, reliable data for the volume of each lake are not available. However, the five lakes have a similar mean depth of ~ 1 m at high tide, making the area of each lake a convenient relative measure of lake size. Lake Cooroibah has the largest catchment area of all the lakes and also has a tidal lake in its upper catchment, which was not included in the catchment area. According to AIC values, Lake Cooroibah was estimated to be the least urbanised of all the lakes, followed sequentially by Lakes Weyba, Cobaki, Terranora and Coombabah (Table 3.1).

Potential 'noise' or variation that natural parameters, such as temperature and salinity may have added to the indicators responses was minimised by selecting lakes with similar assemblages, salinity, depth and tidal ranges. It was difficult to avoid some small latitudinal differences, which may have affected temperature. However, the different levels of urbanisation were interspersed throughout the latitudinal range (26°21' - 28°12') of the lakes.

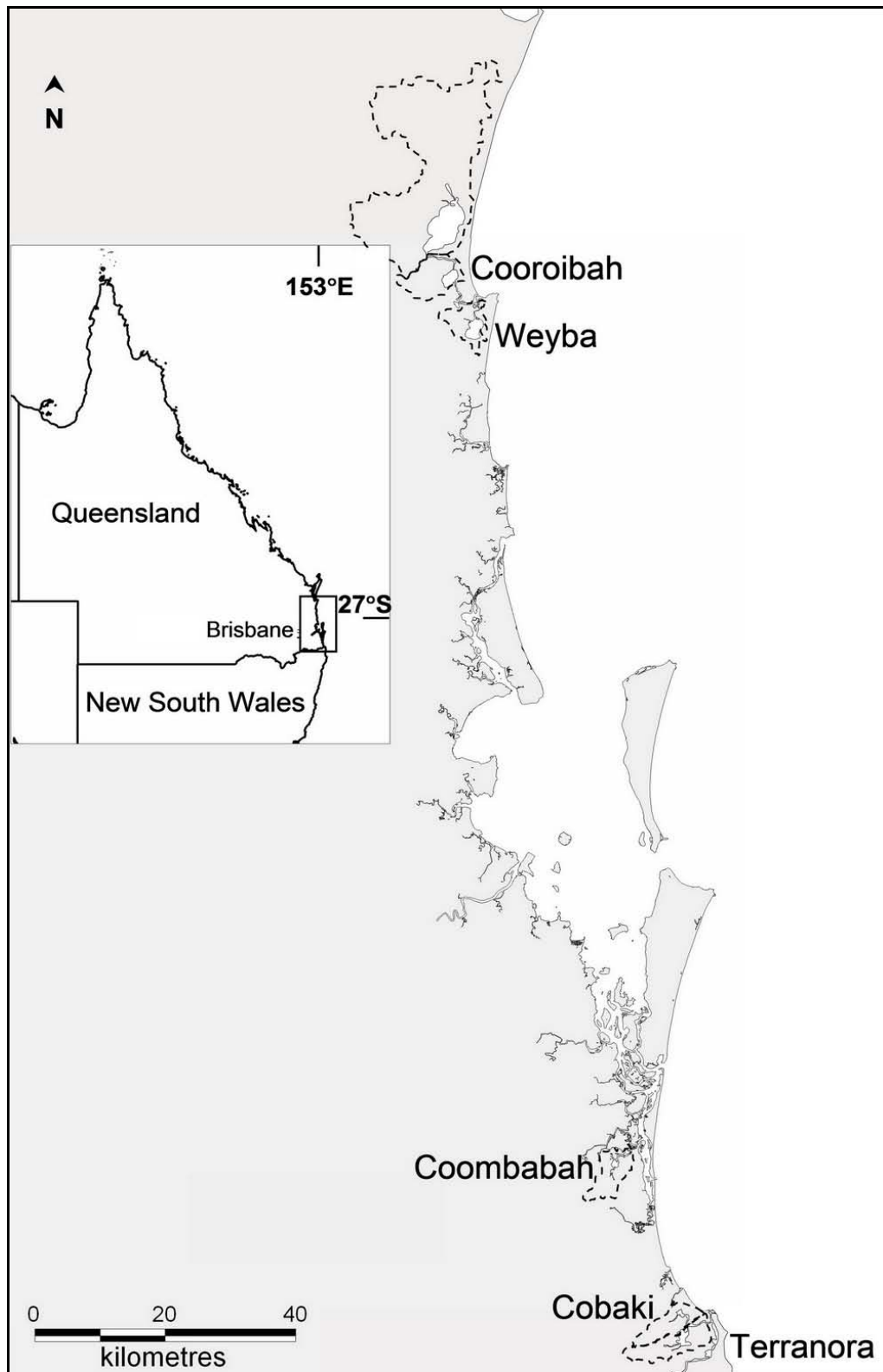


Figure 3.1. Map of southeast Queensland showing the locations of the five estuarine lakes and their catchments (broken lines).

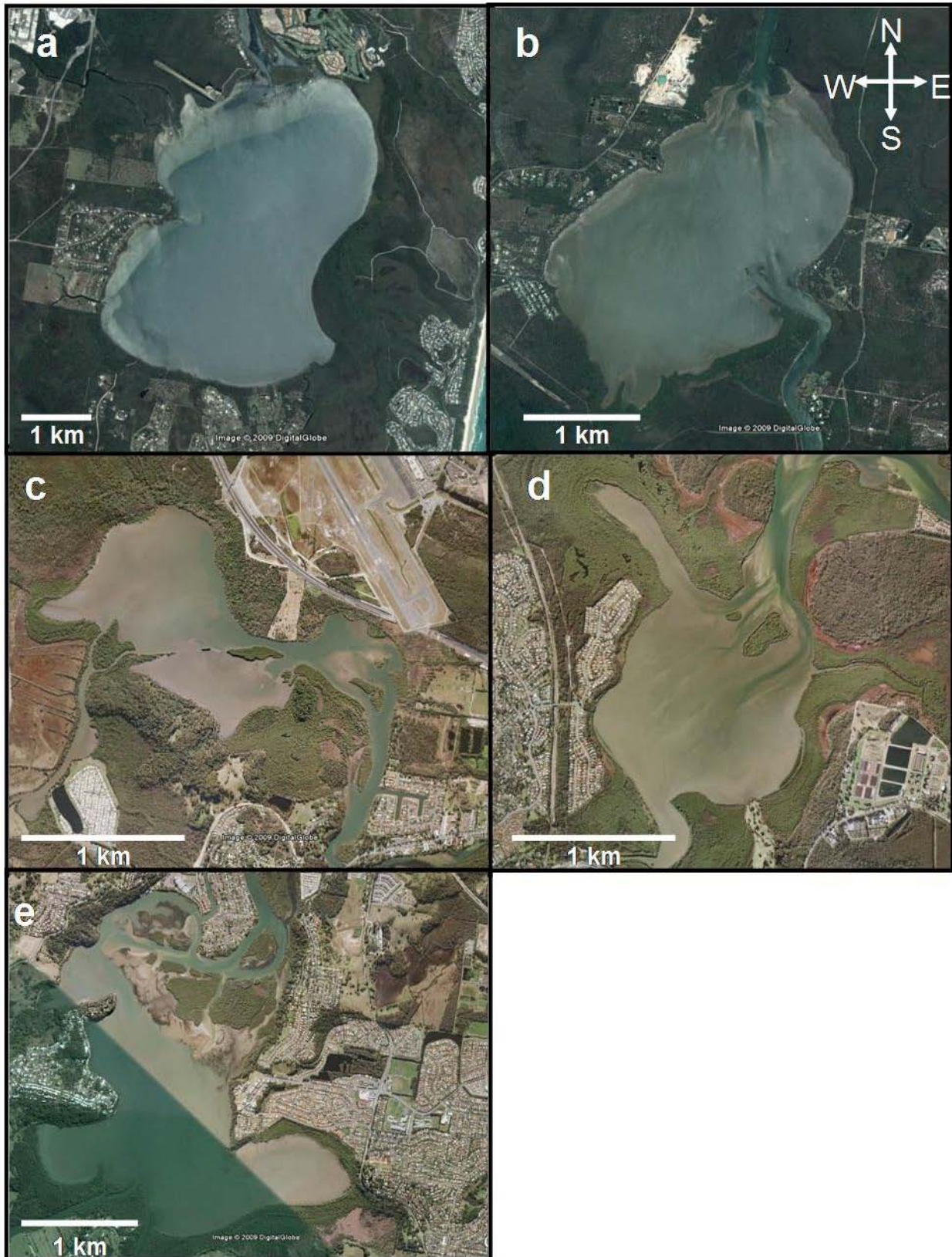


Figure 3.2. Aerial images of lakes: a) Weyba, b) Cooroibah, c) Cobaki, d) Coombabah, and e) Terranora. Reproduced from GE (2008).

Table 3.1. Characteristics of each lake and catchment, ordered by adjusted impervious cover. Land uses are shown as a percentage of the catchment area. CA = catchment area, LA = lake area, IC = impervious cover and AIC = adjusted impervious cover.

Lake	Conservation & natural environments	Agriculture & production from natural environments	Industrial	Rural residential	Urban residential	Water environments	Mining/ waste	CA (ha)	LA (ha)	IC (%)	AIC (%)
Cooroibah	49	29	0	10	2	10	0	65 687	536	0.3	33.1
Weyba	36	18	0	9	14	23	0	3 743	997	9.7	36.4
Cobaki	15	55	1	5	13	7	5	4 870	160	4.2	127.2
Terranora	24	52	0	4	16	3	1	6 400	421	12.4	188.1
Coombabah	23	7	0	0	43	20	7	5 158	208	23.8	591.3
<b>Source</b>			(ABRS 2005)					(EPA 2006b)		(This study)	

### 3.3.2 Sampling design and indicator collection

Samples ( $n = 10$ ) were collected haphazardly from the intertidal mangrove forest of each lake during diurnal low tide over a one-week period. Sampling was replicated six times in total, three times during the wet season (28 Oct 2005 – 27 Mar 2006) and three times during the dry season (5 Jun 2006 – 6 Oct 2006). At all times the following samples were collected from each lake: 10 mangrove leaves, one from each of 10 individual trees (*Avicennia marina*) was collected, as this species was the most prolific and easiest to obtain from all of the lakes); 10 male tuxedo crabs, and red-fingered crabs, 8–20 mm carapace width (CW) were caught and immediately euthanised in an ice slurry. Female crabs were not used because egg production is likely to increase variation in the HI (Kyomo 1988); 10 cores of surface sediment samples (1 cm deep, surface area of 5.7 cm<sup>2</sup>) were collected for measurement of Chl *a*, as an estimate of MPB abundance. Cores were wrapped in aluminium foil and put on ice with the other indicator samples before freezing at -20°C in the laboratory for later analysis. These samples were used to generate a mean value for each type of indicator from each lake at each of the six sampling times.

### 3.3.3 Rainfall

Rainfall data recorded within the vicinity of the lakes between October 2005 and October 2006 were provided by the Australian Bureau of Meteorology. These data were used to estimate when stormwater inputs were likely to enter the lakes. Most likely lag times for each of the indicators' responses to stormwater inputs were based on the response times of similar indicators, as described in Chapter 2 (Table 2.2). These were applied to the rainfall data before testing for correlations with each of the indicators.

### 3.3.4 Measurement of $\delta^{15}\text{N}$ , Chl *a*, and HI

The muscle and hepatopancreas tissues of *Parasesarma erythrodactyla* and the muscle tissues of *Australoplax tridentata* were carefully removed from the crabs. These tissues and the sediment, and mangrove samples were prepared, and  $\delta^{15}\text{N}$ , HI and Chl *a* values were calculated as described in Chapter 2. Prawn was used as a working standard for crab  $\delta^{15}\text{N}$  samples and flour as a working standard for mangrove  $\delta^{15}\text{N}$ . The standard deviation for flour ( $n = 10$ ) was less than  $\pm 0.2\text{‰}$  and less than  $\pm 0.2\text{‰}$  for prawn ( $n = 12$ ).

### 3.3.5 Composite biotic condition index (BCI)

Indicators which displayed a significant response to IC, ( $r \geq 0.50$ ) and were correlated with each other were considered for use in the BCI. These indicators were standardised by dividing their mean value for each level of IC by their largest mean value amongst all levels of IC. Indicators to be used in the final BCI were then confirmed by examining coefficients generated by principal components analysis (PCA) of their standardised values (Bilkovic & Roggero 2008). Indicators that contributed to 30% or more of the variability, accounted for by the first principle component (PC1), were combined into the final BCI. This was done by multiplying the standardised values for each indicator by their respective PC1 coefficients and then summing these values. This gave each indicator a weighting in the final BCI, based upon their contribution to the variability of the multiple indicator data set. A worked example is shown below.

1. Indicators that displayed similar responses to IC were standardised by dividing their mean values for each level of IC by their maximum mean value:

IC (%)	Mangrove $\delta^{15}\text{N}$ (‰)	Crab HI (%)	Chl <i>a</i> ( $\text{mg m}^{-2}$ )
2.5	3.0	4.5	80
5	3.2	3.9	65
11	4.0	3.0	45
18	4.7	2.5	35
25	5.2	2.0	25



Standardised Mangrove $\delta^{15}\text{N}$	Standardised Crab HI	Standardised Chl <i>a</i>
$3.0 \div 5.2 = 0.6$	$4.5 \div 4.5 = 1.0$	$80 \div 80 = 1.0$
$3.2 \div 5.2 = 0.6$	$3.9 \div 4.5 = 0.9$	$65 \div 80 = 0.8$
$4.0 \div 5.2 = 0.8$	$3.0 \div 4.5 = 0.7$	$45 \div 80 = 0.6$
$4.7 \div 5.2 = 0.9$	$2.5 \div 4.5 = 0.6$	$35 \div 80 = 0.4$
$5.2 \div 5.2 = 1.0$	$2.0 \div 4.5 = 0.4$	$25 \div 80 = 0.3$



2. Indicators used in the final BCI were given a weighting based on their contribution to the variability accounted for by the PC1 for the multiple indicator dataset. These values were then summed to give the final BCI:

IC (%)	Std. $\delta^{15}\text{N}$	Weighted $\delta^{15}\text{N}$	Std. Crab HI	Weighted HI	Standardised Chl <i>a</i>	Weighted Chl <i>a</i>
2.5	0.6	$-0.3 \times 0.6 = -0.2$	1.0	0.3	1.0	0.3
5	0.7	-0.2	0.9	0.3	0.8	0.2
11	0.8	-0.2	0.7	0.2	0.6	0.2
18	0.9	-0.3	0.6	0.2	0.4	0.1
25	1.0	-0.3	0.4	0.1	0.3	0.1
PC1 Coefficient:	-0.3		0.3		0.3	



IC (%)	Final BCI
2.5	$-0.2 + 0.3 + 0.3 = 0.4$
5	$-0.2 + 0.3 + 0.2 = 0.3$
11	$-0.2 + 0.2 + 0.2 = 0.2$
18	$-0.3 + 0.2 + 0.1 = 0.0$
25	$-0.3 + 0.1 + 0.1 = -0.1$

### 3.3.6 Data analyses

The indicator samples ( $n = 10$ ) collected from each lake during each of the six sampling times were used to obtain six mean indicator values for each lake. These data were checked for normality and homogeneity of variance using Komolgorov-Smirnov and Levene's tests, respectively. Where necessary, data were natural-log transformed in order to meet these assumptions. Data were also checked for linearity with each of the covariates, AIC and IC, by inspecting scatter plots for each indicator.

Where linear relationships were evident between the indicator values and the covariates, two-factor nested ANCOVA were used to test for any main or interactive effects of Season (Se), Sampling Time nested within Season Ti(Se), and relationships between each covariate, IC and AIC, and the indicators. Where Se and Ti(Se) had no significant effects, mean indicator values were averaged across the six sampling times to obtain one mean value for each indicator (total  $n = 60$  for each mean) from each lake. These mean values were then further tested for relationships with the urban indices (IC & AIC) using regression analyses. If Se or Ti(Se) had any significant effect on relationships between the urban indices and indicators, regression analyses were carried out separately for each level of these variables that

differed. Where scatter plots indicated no relationship between indicator values and the covariates, two-factor nested ANOVA were used to test for any main or interactive effects of Se and Lake (La) on the mean indicator values for each lake.

Correlations between rainfall and mean crab  $\delta^{15}\text{N}$ , HI and Chl *a* measured during each sample time ( $n = 6$ ) in each of the lakes were tested using Pearson's correlation. The correlation coefficients were then tested for relationships with IC and AIC using regression analyses. The temporal variability of the indicators was measured using the coefficient of variation (CV) of the grand mean value for each indicator between successive sampling times within each season. Coefficients of variation (CV, %) provide a measure of variation standardised by the mean, calculated as  $(\text{SD}/\text{mean}) \times 100$  (Quinn & Keough 2002). One-factor ANCOVA were used to test for any effect of Se before combining the mean CV values of each indicator into a mean value for each lake. Mean CV values for each indicator were then tested for relationships with IC and AIC using regression analyses.

### 3.4 Results

#### 3.4.1 Assimilation of urban N

Mean mangrove and crab  $\delta^{15}\text{N}$  values showed no linearity with IC or AIC (Two-factor nested ANCOVA: df 1, 2; all  $p > 0.05$ ). Instead, an *a posteriori* ANOVA test showed that the  $\delta^{15}\text{N}$  values of the indicators were more enriched in lakes which had STPs located within their catchment, or septic tank systems near their shoreline (SNK test, all  $p < 0.05$ , one-factor ANOVA df 4, 25, all  $p < 0.05$ , Figure 3.3).

#### 3.4.2 Algal biomass and crab condition

Mean Chl *a* and crab HI values from all sampling times showed significant responses to AIC (Table 3.2). Season and sampling time had no main or interactive effects on these responses (Table 3.2). Rather than showing a linear decline with urbanisation (Linear regression:  $p > 0.05$ ), Chl *a* exhibited a significant power relationship with AIC (Power regression:  $r^2 = 0.82$ , df 1, 3,  $p = 0.035$ , Figure 3.4). Crab HI values showed negative responses to AIC but there was no significant regression relationship between these indicators and AIC, (Power & linear regressions: all  $p > 0.05$ ). This may have been due to a lack of power, which was limited by the number of field sites available for this experiment. In order to reduce the risk of making a Type II error  $\alpha$  was increased to 0.01. This yielded a significant negative relationship between the HI in tuxedo crabs and AIC, (Power regression:  $r^2 = 0.72$ , df 1, 3,  $p = 0.069$ , Figure 3.4). However the pattern of response in the HI of red-fingered crabs to AIC was too variable to show a significant regression relationship (Power & linear regressions: all  $p > 0.05$ ). Crab condition decreased more with urbanisation in tuxedo crabs than in red-fingered crabs with mean HI values reducing from  $5.0 \pm 0.8\%$  to  $2.0 \pm 0.2\%$  in the former and from  $5.6 \pm 0.4$  to  $3.3 \pm 0.2\%$  in the latter. Mean Chl *a*, and crab HI values showed no significant relationship with IC (linear and power regressions: all  $p > 0.05$ ).

Table 3.2. Significance values for a two-factor nested ANCOVA examining the effects of Season (Se), Time nested within season, Ti(Se) and the covariate, Adjusted IC (AIC) on mean Chl *a* and mean HI values in tuxedo, and red-fingered crabs. Tuxedo crab HI data were natural-log transformed before analysis. The homogeneity of variance assumption was satisfied for all indicators. p values shown in bold denote significance where  $\alpha = 0.05$ .

Source	Chl <i>a</i>				Tuxedo crab HI			Red-fingered crab HI		
	df	MS	<i>F</i>	p	MS	<i>F</i>	p	MS	<i>F</i>	p
AIC	1	4809.77	11.87	<b>0.003</b>	0.79	7.28	<b>0.015</b>	7.11	5.27	<b>0.034</b>
Se	1	87.89	0.21	0.668	0.02	0.30	0.612	0.25	0.47	0.532
Ti(Se)	4	410.19	1.01	0.427	0.07	0.69	0.611	0.53	0.39	0.810
Se $\times$ AIC	1	18.84	0.05	0.832	0.00	0.01	0.922	0.58	0.43	0.521
Ti(Se) $\times$ AIC	4	181.44	0.45	0.773	0.06	0.51	0.731	0.20	0.15	0.962
Residual	18	405.19			0.11			1.35		

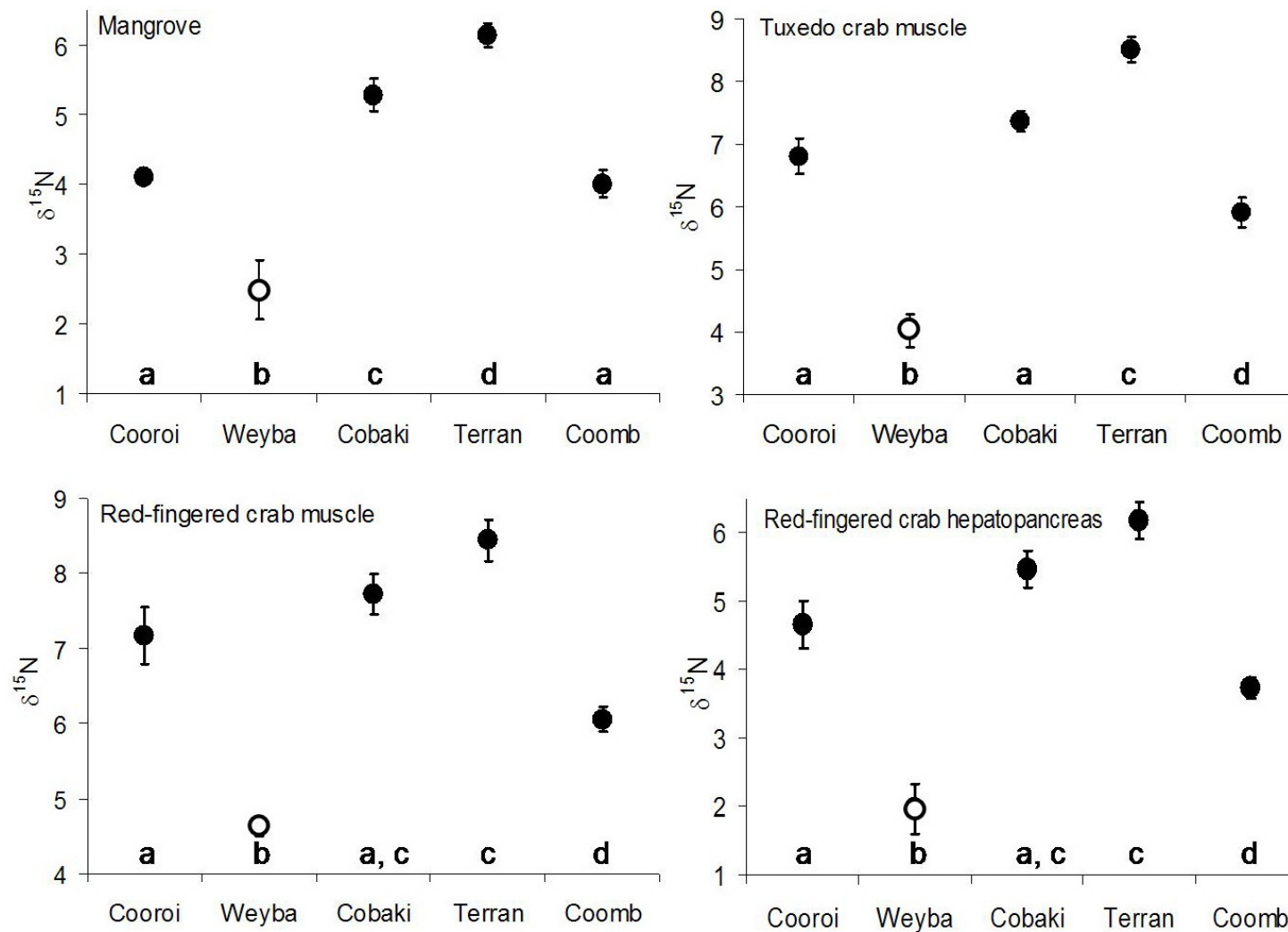


Figure 3.3. Mean ( $\pm$  SE) mangrove and crab  $\delta^{15}\text{N}$  values in Lakes Cooribah, Weyba, Cobaki, Terranora, and Coombabah, averaged across sampling times ( $n = 6$ ) during October 2005 – 2006. Black symbols indicate lakes with potential exposure to isotopically enriched N from urban sewage (STPs or septic tanks). Different letters (a, b) indicate significant differences in isotope values between lakes.

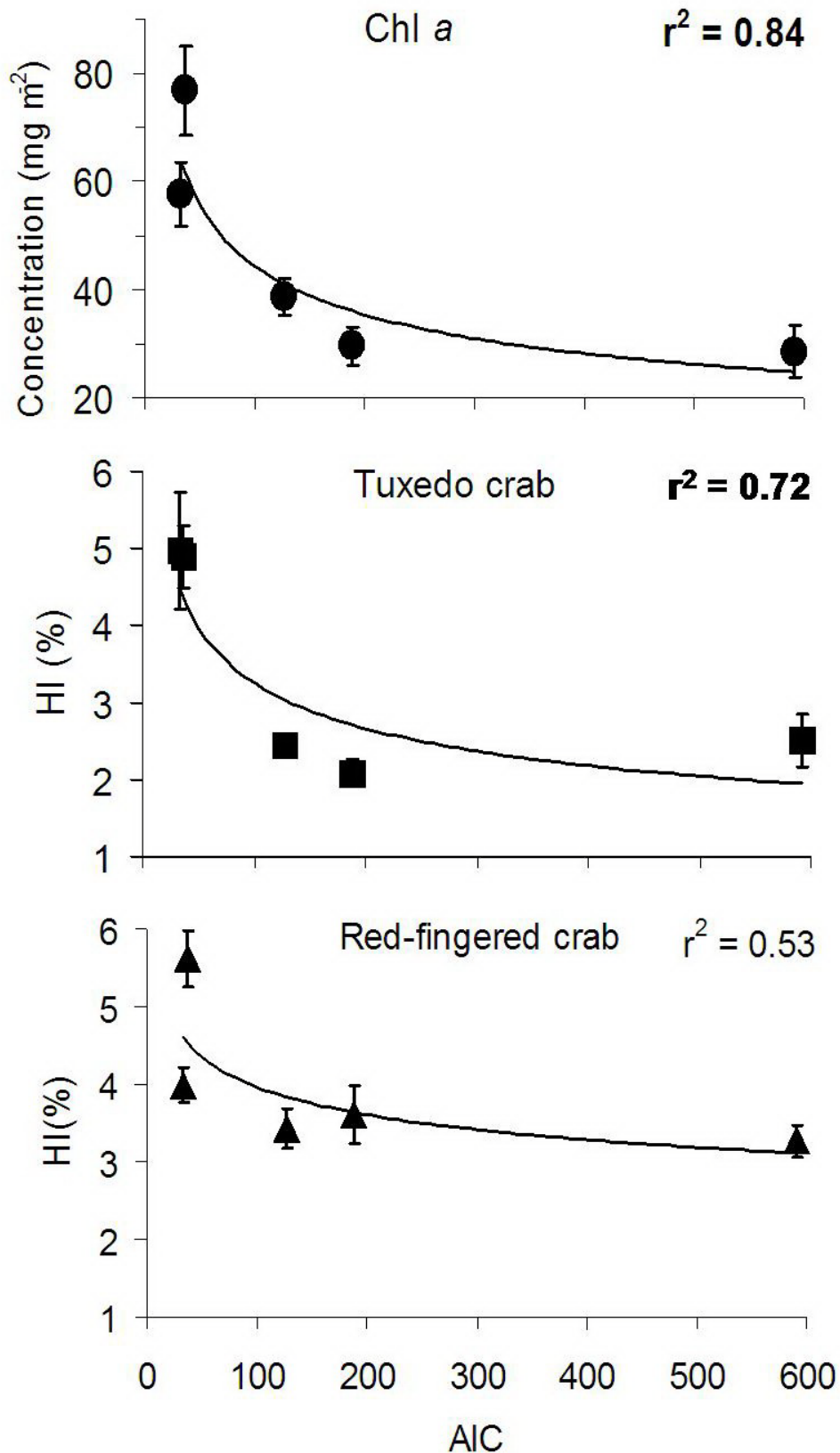


Figure 3.4. Responses of mean ( $\pm$ SE): a) Chl *a*; b) tuxedo crab HI and; c) red-fingered crab HI to AIC. Significant  $r^2$  values are shown in bold (Chl *a*:  $p < 0.05$  and Tuxedo crab HI:  $p < 0.1$ ). Each data point is the mean of mean indicator values ( $n = 10$ ), measured during six sample times.

### 3.4.3 Composite index of biotic condition (BCI)

Because  $\delta^{15}\text{N}$  values in crabs and mangroves showed no significant response to IC or AIC, or any correlation with the other indicators (Pearson's test: all  $p > 0.05$ ), they were not included in the final BCI. Mean Chl *a*, tuxedo crab, and red-fingered crab HI values all displayed either significant regression or covariate responses to AIC; these responses were also correlated amongst the three indicators (Pearson's test: All  $r > 0.90$  and  $p < 0.05$ ). Principal component analysis showed that Chl *a*, and crab HI, in both species, had similar loading values, and were positively associated with the first principal component (PC1), which explained 91% of the variance amongst these indicators (Table 3.3). Based on their responses to AIC, correlation with each other, and contribution to the PC1, the mean Chl *a* and HI values of both crab species were combined into the final BCI. The relationship between the BCI and urbanisation (AIC) was best described by a negative power curve, with a steep decline in BCI between AIC values of 37 and 127 (Power regression:  $r^2 = 0.79$ ,  $df = 1, 3$ ,  $p = 0.045$ , Figure 3.5).

### 3.4.4 Temporal variation

Coefficients for correlations between Chl *a*, crab  $\delta^{15}\text{N}$ , crab HI and rainfall did not show any significant response to IC or AIC (Regression: all  $p > 0.05$ ). The mean temporal variation (CV) of red-fingered crab muscle  $\delta^{15}\text{N}$  responded interactively to IC between seasons (One-factor ANCOVA:  $df = 1, 16$ ,  $F = 4.55$ ,  $p < 0.05$ ). However, separate analyses of this indicator for each season showed no significant relationship with IC during either season (Linear & power regressions: both  $p > 0.05$ ). Mean temporal variance (CV) values showed no significant response to IC or AIC in any of the other indicators (One-factor ANCOVA: all  $p > 0.05$ ).

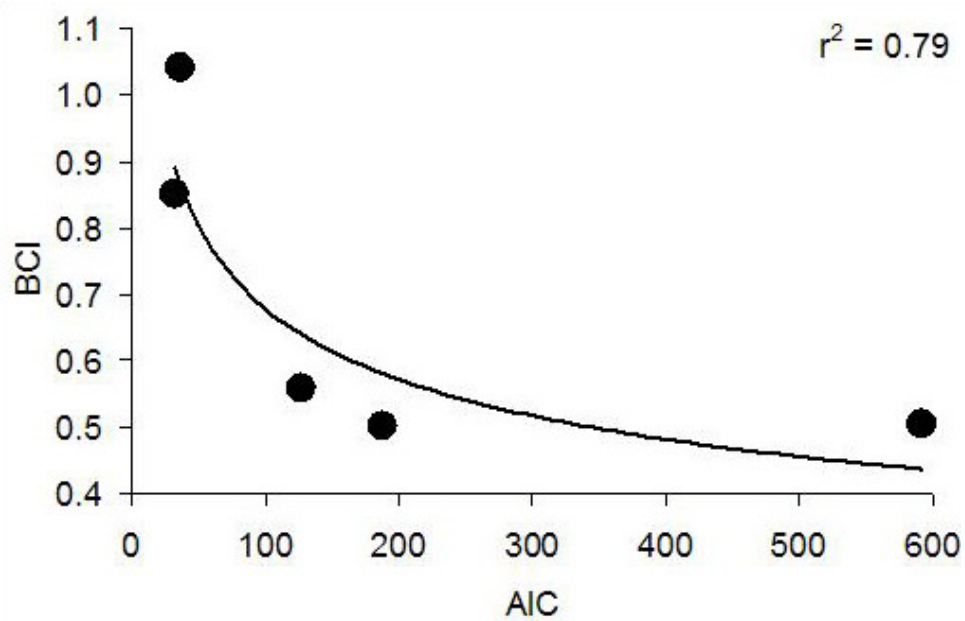


Figure 3.5. Relationship between the biotic condition index (BCI) and AIC ( $p < 0.05$ ).

Table 3.3. Coefficients and accountable variance of the first principal component based on individual biotic indicators.

Indicator	PC1
Chl <i>a</i>	0.36
Tuxedo crab HI	0.34
Red-fingered crab HI	0.34
% variance accounted for	91.25



### 3.5 Discussion

#### 3.5.1 Assimilation of isotopically enriched N from potential urban sewage sources

Mangrove and crab  $\delta^{15}\text{N}$  values showed no relationship with IC, or AIC, thus rejecting the hypothesis that the assimilation of urban N by estuarine biota would increase with urbanisation. This response indicates that urban runoff delivered via impervious surfaces is not the dominant source of urban N in the lakes. Instead, the pattern of enrichment in mangrove and crab  $\delta^{15}\text{N}$  indicates that STPs and septic tanks located within the lakes' catchments are the predominant sources of urban N (e.g., Savage & Elmgren 2004, Steffy & Kilham 2004). There is no direct release of treated effluent into any of the lakes, therefore groundwater seepage from damaged sewer lines and poorly maintained septic tank systems, or tidal advection from effluent released downstream are the only likely means of conveyance of enriched  $\delta^{15}\text{N}$  sources into the lakes. Agricultural activities, which produce animal waste, are present in some of the catchments. These may have also contributed to the assimilation of enriched  $\delta^{15}\text{N}$  sources (Heaton 1986).

Mangrove and crab  $\delta^{15}\text{N}$  values were most enriched in Lakes Terranora and Cobaki. Both of these lakes have STPs located within their lower catchments, which discharge tertiary-treated wastewater ~ 2 km downstream during the ebbing tide (McMahon 2005). The Banora Point STP located ~2 km downstream from Lake Terranora treats approximately  $10.8 \text{ ML day}^{-1}$  and the Tweed Heads West STP, located 500 m east of Lake Cobaki releases ~  $2.4 \text{ ML day}^{-1}$  (McMahon 2005). Previous research indicates there is no upstream advection of N from the effluent discharge point into either of the lakes and that the water quality of Lake Terranora is predominantly driven by urban runoff (McMahon 2005). However, some degree of connectivity may exist between the actual Tweed Heads West STP and Lake Cobaki due to their close proximity. A drainage line connecting the STP to the lower reaches of Lake Cobaki makes it possible for N derived from sewage to enter the lake. Due to its close proximity to the lake, the STP is also likely to contribute enriched  $\delta^{15}\text{N}$  via groundwater flows, particularly when connectivity between groundwater surrounding the plant and Lake Cobaki is increased by rainfall and spring high tides. Groundwater transport of nutrients from urban sewage into estuaries such as Waquoit Bay, US, have been well documented (e.g., Valiela et al. 1997).

The Banora Point STP is located farther away from Lake Terranora (~2 km downstream), therefore connectivity between this STP and the lake is unlikely (McMahon 2005). Although urban runoff is considered the predominant source of N in this lake, no

studies have explicitly traced this linkage and it is possible that a number of sewage sources within the catchment also contribute N to the lake. A number of residences located around the shores of Lake Terranora, however, have onsite septic tanks. Due to their close proximity to the lake, these systems are likely to leach enriched sewage  $\delta^{15}\text{N}$  into Lake Terranora via groundwater flows. Urban stormwater has also been identified as a principal source of N in Lake Terranora and may have also contributed to the enriched mangrove and crab  $\delta^{15}\text{N}$  values in the Lake (Anon 1998, McMahon 2005, Dillon & Chanton 2008). Lake Cobaki and Lake Terranora also have substantial areas of farm land (> 50%) within their catchments. Therefore, effluent from cattle and pig farming activities within the catchments may also be a source of isotopically enriched N in these lakes (Heaton 1986). Bio-solids from the STPs, which are re-used as soil conditioner in some of these agricultural areas, are also likely to contribute enriched  $\delta^{15}\text{N}$  to the lakes (McMahon 2005).

Mangrove and crab  $\delta^{15}\text{N}$  were not as elevated in Lake Cooroibah and Lake Coombabah, but had more enriched values than those expected for pristine estuarine environments (e.g. mangrove  $\delta^{15}\text{N} \sim 2\text{‰}$ , Costanzo et al. 2001) indicating some assimilation of urban N (McClelland et al. 1997, Costanzo et al. 2001, Savage & Elmgren 2004, Dillon & Chanton 2005). Many of the lakeside residences around Lake Cooroibah have onsite septic tanks, which may leach enriched  $\delta^{15}\text{N}$  into the lake in groundwater flows (McClelland et al. 1997, NSC 2007). Approximately 14% of the Cooroibah catchment is designated for farming and agricultural use, therefore N from animal effluent in these areas may have made some contribution to the enriched  $\delta^{15}\text{N}$  values of biota in the Lake (Heaton 1986). Coombabah is the most urbanised of the lakes but biota within this lake did not have the most enriched  $\delta^{15}\text{N}$ , indicating that the extent of catchment urbanisation (AIC) is not the most important factor contributing to the assimilation of urban N in the lakes. As indicated in Chapter 2, the STP located on the lake's eastern shore is probably the dominant source of enriched  $\delta^{15}\text{N}$ . However, urban runoff may contribute some N sources to the lake following rainfall (e.g. Dillon & Chanton 2008).

The depleted crab and mangrove  $\delta^{15}\text{N}$  values within Lake Weyba indicated an assimilation of predominantly natural N sources (Heaton 1986). Most of the urbanisation within the Weyba catchment is located downstream of the lake. These residences are connected to mains sewerage, which is treated at the Noosa STP, located outside of the Weyba catchment. The rural residential houses surrounding the rest of the lake use septic tanks (NSC 2007). However, most of these houses are set well back (at least 100 m) from the lake, with natural

vegetation separating them from the lake in most areas. This urban design is likely to reduce any leakage of N from on site septic systems into the lake. The depleted  $\delta^{15}\text{N}$  values of biota within Lake Weyba may also indicate an assimilation of N from synthetic fertilisers applied to land used for agricultural purposes or the golf course at the northern end of the lake (Heaton 1986). The only form of agricultural activity within this catchment, however, is grazing on natural vegetation and the use of synthetic fertilisers for this type of agriculture is likely to be minimal.  $\delta^{15}\text{N}$  values did not show any localised depletion in biota collected from sites near the golf course, indicating that there is no obvious difference in nutrient sources between this end of the lake and its more pristine areas. Therefore, the depleted  $\delta^{15}\text{N}$  values observed in Lake Weyba are more likely to reflect the assimilation of natural N sources.

### **3.5.2 MPB biomass and crab condition form a composite index of biotic condition**

MPB biomass and crab condition displayed similar negative responses to AIC and were combined to form the BCI. MPB biomass showed the strongest relationship with AIC and due to its strong correlation with crab condition, some would suggest its independent use as a surrogate indicator of BCI. In this study, however, combining the responses of MPB and crab condition provided a better insight to a trophic cascade of urban impact between benthic primary producers and consumers. In this instance, the combination of these variables into the BCI provided a convenient, easily communicable indicator, which was more representative of the ecosystem level of response to urbanisation.

The condition of tuxedo crabs showed a stronger negative relationship with AIC than the condition of the red-fingered crabs. The decline in condition in tuxedo crabs may have been more closely linked with the decline in MPB biomass due to a greater dependence on this food source by this species. Previous studies have indicated that ocypodid crabs such as the tuxedo crab rely more heavily upon MPB as a food source (Rodelli et al. 1984, Oakes 2006). Whereas the red-fingered crabs are thought to derive their energy from a broader range of sources including MPB, mangrove and saltmarsh detritus (Camilleri 1989, Werry & Lee 2005, Oakes 2006). This species has also been observed to eat other crab species (pers. obs.). This more generalist approach to foraging is likely to dampen any responses in the condition of this species associated with MPB patterns.

#### **3.5.2.1 *Biotic condition shows a negative relationship with adjusted IC.***

The BCI showed a significant negative relationship with AIC but showed no obvious response to IC. This indicates that in estuarine lakes AIC provides a better proxy for biotic responses to urbanisation than IC. Previous studies have demonstrated IC as a reasonable

predictor of urban impacts in receiving estuarine creeks, without being adjusted for the sensitivity (catchment to estuary size ratio) of these systems (e.g., Sanger et al. 1999a, b, Holland et al. 2004). The sites examined in these studies, however, were well-flushed macro-tidal creeks where tidal flushing is likely to play an important role in the dilution of urban inputs (Dame et al. 2000, Van Dolah et al. 2008). The estuarine lakes assessed in this study have smaller tidal ranges and are likely to have considerably longer water residence times, enabling a greater accumulation of urban pollutants through time (Soballe & Kimmel 1987). Therefore, water volume is likely to play a more important role in the dilution of pollutants in estuarine lakes than in macro-tidal creeks. This indicates that the catchment to estuary size ratio has a greater influence on the responses of estuarine lakes to catchment disturbance than in well-flushed estuarine systems.

The response in biotic condition to increased urbanisation was best explained as a power relationship, with rapid declines in BCI occurring between AIC values of 37 and 127. A greater replication of points on this regression would have given a more precise estimate of the AIC range at which biota start to respond significantly, however it was not possible to achieve this without encountering confounding factors associated with latitudinal differences between sites, as well as exceeding the range of indicator species distributions. Nonetheless, biota sampled from the five lakes indicated a power shaped decline in condition with greater AIC. This power shaped decline in BCI may reflect how differences in the sensitivity of specific systems influence this relationship. The abrupt decline in condition occurred in Lake Cobaki, which had the second highest catchment to lake area ratio and only 4.2 % IC. Lake Weyba, which had 9.7% IC, had the highest BCI, but the smallest catchment to lake area ratio, making it the least sensitive of all the lakes. This response supports the model that smaller estuarine lakes with larger catchments are more sensitive to catchment urbanisation (Horton & Eichbaum 1991). Lake Weyba has a sandier substrate than the other four lakes, which may have also influenced the high BCI observed in this lake and hence, the large difference in BCI between Lake Weyba and Lake Cobaki. Lake Cooroibah, however, has a similar muddy substrate to the other, more urbanised lakes and also had a considerably higher BCI level than these lakes, thus supporting the model that greater catchment urbanisation explained most of the decline in biotic condition.

Differences between the lakes in the extent of drainage connectivity with impervious surfaces may have also contributed to the power shape of the relationship. Reductions in ecosystem health are likely to be more rapid in systems with greater drainage connection to impervious surfaces, as these systems receive more urban pollutants than those with less direct

drainage from impervious areas (Walsh 2000). These types of system specific factors should be considered when applying IC as a guide for urban impact thresholds in receiving estuaries.

### **3.5.2.2 *How is IC reducing the condition of biota in the lakes?***

The negative response in BCI indicates that impervious surfaces convey pollutants into the lakes that impair the biomass and condition of estuarine biota. This result indicates that benthic primary production and consumer condition are not being subsidised by urban nutrients. Alternatively, nutrients delivered in urban runoff may have reduced the growth of submersed MPB by enhancing the growth of phytoplankton in the water column, and increasing the level of benthic shading (Meyercordt & Meyer-Reil 1999). This idea is supported by the observation of elevated Chl *a* levels in the water column of Lake Coombabah ( $6.2 \pm 1.0 \mu\text{g L}^{-1}$ ), the most urbanised of the lakes, compared to in Lakes Cooroibah ( $2.3 \pm 0.4 \mu\text{g L}^{-1}$ ), and Weyba ( $1.9 \pm 0.3 \mu\text{g L}^{-1}$ ), the least urbanised of the lakes (GCCC 2004, EPA 2005a). However, the intertidal MPB measured in this study is typically only covered by ~ 30 cm of water during high tide and can receive up to 6 hours of direct sunlight during daytime low tides, when MPB fulfil most of photosynthetic requirements (Pinckney & Zingmark 1991). Therefore, any effects of shading associated with increased phytoplankton growth are probably negligible. Furthermore, the enriched  $\delta^{15}\text{N}$  values observed in crabs and mangroves showed no correlation with the response in MPB biomass, indicating that the decline in MPB was more likely a response to pollutants other than urban nutrients.

Sediment loads conveyed into the more urbanised lakes in urban runoff may have contributed to the decline in MPB. Greater turbidity levels have been recorded in Lake Coombabah ( $25.3 \pm 5.6 \text{ ntu}$ ), the most urbanised of the lakes, than in Lakes Cooroibah ( $15.1 \pm 1.8 \text{ ntu}$ ) and Weyba ( $9.4 \pm 2.75 \text{ ntu}$ ), adding support to this idea (GCCC 2004, EPA 2005a). Greater sediment loads can reduce benthic primary production in a number of ways. The erosive forces of increased suspended sediments may increase the resuspension of diatoms living near the sediment surface, resulting in a greater export of MPB from intertidal flats to deeper channels during ebb tide flows (MacIntyre et al. 1996, Lucas 2003, Guarini et al. 2004, Pinckney & Lee 2007).

Greater sediment loads may also lead to long-term increases in turbidity by reducing the size composition of surface sediments and making them more susceptible to resuspension (MacIntyre et al. 1996, Anderson et al. 2004). Such increases in turbidity can reduce the photosynthetic rate of MPB, due to reductions in light penetration to the benthos (MacIntyre et al. 1996). However, as mentioned earlier, this effect is likely to small due to the shallow (~ 30

cm) inundation of the mudflats from which the MPB was collected in this study. Admiraal & Peletier (1980) documented that increased turbidity only reduced the growth rate of MPB located in the lower intertidal zone. Furthermore, sediments which have a high mud content can be very cohesive, which can make them less susceptible to resuspension than some coarser sediments. Therefore, the idea that increased loads of fine urban sediments lead to long term increases in turbidity needs justification.

The accumulation of fine terrestrial sediments in urbanised estuaries may initially increase the settlement rate of juvenile macrofauna which require soft substrate for recruitment. Anderson et al. (2004) reported an increase in the density of burrowing crabs in high-deposition areas of an estuary. Increases in grazing pressure and bioturbation associated with greater densities of crabs may have also contributed to the decline in MPB biomass (Taylor & Allanson 1993, Armitage & Fong 2006). If this scenario is true, then increased sedimentation may also lead to greater rates of competition between crabs in urbanised estuaries, thus potentially explaining the negative trend in crab condition with increased urbanisation. The assessment of crab density, sediment properties and competition rates may help to improve our understanding of these potential ecosystem level responses to sedimentation associated with urbanisation.

Pollutants other than nutrients and sediments may have also contributed to the decline in biotic condition. Urban runoff typically contains a mixture of metals, petroleum hydrocarbons, herbicides, sediments, nutrients, pathogens such as faecal coliform bacteria and rainwater (Drapper et al. 2000, Mallin et al. 2000, Gobel et al. 2007). Local councils also spray insecticides over saltmarsh areas surrounding estuaries in urbanised areas, in order to control mosquitoes. Many of these pollutants bind to the sediments in receiving estuaries and accumulate over time (e.g., Abraham et al. 2007). Elevated levels of metals and pesticides have been reported in crabs (*Australoplax tridentata* and *Scylla serrata*) from estuaries adjacent to urban areas of Brisbane (Mortimer 2000). These pollutants have the potential to reduce MPB production, as well as the condition and abundance of consumers (Moreno-Garrido et al. 2003, Elumalai et al. 2005, Ma et al. 2006, Bouilly et al. 2007).

### **3.5.2.3 Implications for estuarine ecosystems**

MPB are thought to be a significant carbon source for many estuarine consumers (Dittel et al. 1997, France 1998, Lee 2000, Bouillon et al. 2002a, Bouillon et al. 2002b, Guest et al. 2004, Bouillon et al. 2008). Thus, the decline in MPB biomass indicates that as urbanisation increases, the ability of mangrove environments to provide this important food source to the

estuarine food web is reduced. The responses of MPB biomass and crab condition indicate a cascade of urban impacts from primary producers to consumers. The decline in MPB biomass with increased urbanisation is likely to have triggered the negative response in crab condition, due to a reduction in the crabs food supply (e.g., Kennish 1997). In addition, urban pollutants absorbed by the MPB are likely to have been passed onto the crabs via ingestion (e.g., Chaufan et al. 2006). As some of these pollutants can accumulate within the crabs over time, their toxic effects are also likely to have contributed to the negative response in crab condition.

The reduction in crab condition is likely to reduce the resilience of individual crabs to additional disturbances, including stochastic events such as floods, sewage overflows or disease outbreaks. Under such circumstances, the survival of the crabs may be reduced, making their populations more vulnerable to local extinction. These crabs contribute importantly to mangrove ecosystems. Their feeding and burrowing activities enhance the biogeochemical cycling of carbon and nutrients, influence mangrove production and maintain the structure of the forest floor (Kristensen 2008, Lee 2008). The crabs themselves are also likely to be an important trophic link between mangrove primary producers and mobile consumers such as fish (Smith 1987, Sheaves & Moloney 2000, Cannicci et al. 2008, Lee 2008). Due to their abundance, these crabs have a substantial influence on the structure and function of mangrove ecosystems, thus impacts on the resilience of these animals could potentially lead to alterations in the resilience of their habitat.

### **3.5.3 Relationship between IC and the temporal variation of biotic indicators**

This study did not support the model that the temporal variation of biotic responses would increase with urbanisation. The temporal variability of crab condition measured in Chapter 2 appeared to provide a sensitive indication of impacts at a localised scale around urban influx sites in Lake Coombabah. In this study however, short-term fluctuations in the indicator values at the ‘whole-lake’ scale may have been diluted by the size of the lakes. The dispersal of pollutants delivered in urban runoff may have been restricted to localised areas around influx points such as stormwater drains and creeks in the lakes. The slow flow rate and large size of the lakes are likely to have prevented urban runoff from dispersing around the whole lake without becoming substantially diluted in the process, therefore making changes in temporal variability difficult to detect at the whole lake scale. Below average rainfall during this study, is also likely to have contributed to the lack of response in temporal variability. Low rainfall would have reduced the intensity of stormwater runoff events into the lakes. Monitoring the temporal variability of biotic indicators over a longer time period is likely to

capture the responses of the estuaries to larger storm runoff events. This may provide a better indication of alterations to the temporal variability in response to increased urbanisation around the lakes.

### **3.5.4 Summary and conclusions**

This study investigated whether a relationship exists between urbanisation (measured as IC) and the condition of biota in five estuarine lakes around southeast Queensland. The  $\delta^{15}\text{N}$  of mangrove biota indicated an assimilation of urban nutrients in all but one of the lakes. This appeared to be associated with the presence of STPs and septic tanks within the lakes' catchments and was not related with the extent of urbanisation surrounding the lakes. MPB biomass and crab condition were combined into a composite index of biotic condition, which showed a negative response to increased urbanisation only when the IC of the lakes' catchments had been adjusted for differences in the ratio of catchment area to lake area. This ratio is thought to influence the sensitivity of estuarine systems to catchment disturbance (Horton & Eichbaum 1991). The decline in biotic condition together with its lack of correlation with the assimilation of urban nutrients by mangrove biota indicates that urban pollutants that inhibit primary production and consumer condition have a greater effect on estuarine biota than urban nutrient loads.

This study offers support for the model that the increased urbanisation of coastal areas around southeast Queensland has an impact on estuarine biota, reducing benthic primary production and the condition of benthic consumers. However, the magnitude of this effect also depends on specific characteristics of the receiving estuary and its catchment, highlighting the need to consider these factors when applying IC as a predictor of urban impacts in estuaries. Further investigation of the relationship between IC and biotic responses in different types of estuarine systems will improve our understanding of this relationship and provide further validation for its use in the management of urbanised coastal areas around Australia.





## Chapter 4. Biotic responses to increased urbanisation around estuarine creeks of southeast Queensland

### 4.1 Abstract

Increasing urban development along coastal areas in southeast Queensland threatens the health of the region's estuaries. As coastal areas become more urbanised a greater proportion of their catchments are covered by impervious surfaces, which concentrate and convey stormwater borne urban pollutants into coastal estuaries. Previous studies, as well as the results from Chapter 3 show an increasing impact on the biota of estuaries with greater catchment imperviousness. This relationship, however, needs broader validation in different types of estuaries.

In a rapidly urbanising coastal area of southeast Queensland, I further assessed this relationship in eight mangrove-dominated estuarine creeks. I tested the model that mangrove biota would become more impacted as the catchment impervious cover (IC) surrounding the creeks increased. This would be shown by a greater assimilation of urban nitrogen (N) (i.e. from sewage and stormwater sources), as indicated by enrichment of  $\delta^{15}\text{N}$  in the grey mangrove (*Avicennia marina*) and the tuxedo crab (*Australoplax tridentata*), and reductions in the biomass of microphytobenthos (MPB) and crab condition. Four of the creeks were also exposed to the release of tertiary-treated effluent from neighbouring sewage treatment plants (STPs). Therefore I also tested whether the presence of STPs had any effect on these indicators.

Biota in creeks with adjacent STPs had significantly enriched  $\delta^{15}\text{N}$  values, indicating an assimilation of urban N in these creeks. The exposure to these urban N inputs, however, had no effect on the MPB biomass or condition of crabs in the creeks. MPB biomass and crab condition showed similar responses to increased IC, indicating a cascade of urban impact between primary producers and consumers. The combination of these two indicators into a composite index of biotic condition (BCI) showed a negative response to increased catchment urbanisation. Linear regression analysis indicated that catchment IC accounted for 61% of the variability in the biotic condition of the creeks.

This study supports the model that increased IC leads to growing impacts on the biota of estuarine creeks. However, differences between these creeks and the estuarine lakes in Chapter 3, including factors that influence the sensitivity of these systems, highlight the need to consider system-specific features when applying IC to estimate biotic responses to urbanisation.

## 4.2 Introduction

The coastline of southeast Queensland encompasses an extensive array of estuarine environments. The region includes all catchments that flow into Moreton Bay Marine Park, a large coastal embayment, which offers extensive estuarine habitats to a diverse assemblage of invertebrate, fish, reptile, bird, and mammal species. Increasing urbanisation of the region's coastal areas however, is placing these valuable ecosystems under pressure. Human settlement in coastal areas has already affected the water quality of many estuaries in the region (EPA 2007). Chapter 3 showed a decline in the biotic condition of estuarine lakes around southeast Queensland as urbanisation in the lakes' catchments increased. This was strongly associated with the impervious cover (IC) of catchments surrounding the lakes, together with the sensitivity of the lakes to catchment influences (ratio between the area of surrounding catchment and the area of each lake).

The proportion of catchment covered by impervious surfaces (IC) has been well correlated with increased housing density, and urban runoff volumes (Schueler 1987, Arnold & Gibbons 1996, Cappiella & Brown 2001). Urban runoff contains a wide variety of pollutants, including heavy metals, nutrients, petroleum hydrocarbons, freshwater and sediments (Sanger et al. 1999a, b, Drapper et al. 2000, Anderson et al. 2004, Holland et al. 2004). The increased volumes of urban runoff, which are channelled into waterways via impervious surfaces can degrade receiving aquatic ecosystems (Walsh et al. 2001, CWP 2003). Most previous research on the relationship between IC and ecosystem responses has, however, been focused on freshwater systems (e.g., Booth & Jackson 1997, Walsh et al. 2001, CWP 2003), with few studies examining the relationship in estuarine systems (e.g., Lerberg et al. 2000, Holland et al. 2004). Distinctive characteristics of these estuaries, including their large tidal range and location, prevent the application of this relationship to estuaries in a broad sense. Further investigation of the relationship between IC and the responses of biota in other types of estuaries will improve our understanding of how IC may be used as a predictor of urban impacts in a broader range of estuaries.

Estuarine creeks are widespread around Moreton Bay and provide extensive mangrove and saltmarsh habitats to many fish and invertebrates (Kneib 1997, Lee 1999, Thomas & Connolly 2001). Due to their high edge to volume ratio and abundance, collectively the role of estuarine creeks in connecting southeast Queensland catchments to Moreton Bay may be as important as that of larger estuarine systems such as rivers and lakes (Dame et al. 2000, Mallin & Lewitus 2004). The close proximity to Moreton Bay and waterfront locations offered within the catchments of estuarine creeks, has encouraged the rapid urbanisation of areas surrounding

these environments. The high edge to volume ratio in estuarine creeks may make these systems more vulnerable to urban inputs than larger systems such as estuarine lakes. In contrast, estuarine creeks are also likely to receive better tidal flushing than estuarine lakes, which may contribute to a greater dilution of urban pollutants in the creeks. In either case, these differences between estuarine lakes and creeks are likely to influence the relationship between IC and estuarine biota.

I investigated the relationship between impervious cover (IC) and the responses of biota in eight estuarine creeks around southeast Queensland by measuring the  $\delta^{15}\text{N}$  of the grey mangrove (*Avicennia marina*) and the tuxedo crab (*Australoplax tridentata*), as a tracer for urban nutrients (N), sediment Chlorophyll *a* concentration (Chl *a*) as a proxy for MPB (microphytobenthos) biomass, and the hepatosomatic index (HI) as a proxy for crab condition. Chapter 3 indicated that estuarine lakes vary in their sensitivity to catchment influences according to differences in the ratio of catchment to lake surface area (Horton & Eichbaum 1991). IC was therefore adjusted for differences in this ratio between the creeks to give an adjusted IC index (AIC), which was also tested as a predictor of urban impact in the creeks. Four of the creeks are within close proximity to sewage treatment plants (STPs). Tertiary-treated sewage is released daily during the ebb tide into the lower reaches of three of the creeks (Hayes Inlet, Cabbage Tree Creek, and Eprapah Creek) and within 1 km of the mouth of Bulimba Creek. I also investigated whether this exposure to sewage inputs had any effect on biota within the creeks.

Based on the biotic responses observed in the previous chapters, I evaluated the model that mangrove biota in estuarine creeks would become more impacted as urbanisation (IC or AIC) increased. This would be demonstrated by an increased assimilation of urban N by mangrove biota, and declines in benthic primary production (MPB biomass) and consumer condition (crab HI) with increased IC or AIC of the catchments surrounding the creeks. Creeks that were exposed to sewage effluent were likely to receive greater loads of urban nutrients, therefore I also tested whether  $\delta^{15}\text{N}$  values, MPB biomass and crab condition increased due to exposure to sewage effluent. Biotic indicators that were related with IC or AIC were combined to provide a composite indicator of biotic condition (BCI). The following hypotheses were tested:

(1) The  $\delta^{15}\text{N}$  values of mangroves and crabs, and MPB biomass and crab condition would be greater in creeks exposed to sewage effluent;

(2) The assimilation of urban (stormwater) N by mangrove biota would increase with urbanisation. This would be demonstrated by enrichment in mangrove and crab  $\delta^{15}\text{N}$  values with increased IC and/or AIC;

(3) The biomass of MPB (Chl *a*) and crab condition (HI) would decrease with increasing IC and/or AIC and;

(4) The BCI formed using the indicators measured in this study would decrease with greater IC and/or AIC.

## 4.3 Methods

### 4.3.1 Study sites and estimation of urbanisation

Eight estuarine creeks located around Moreton Bay, southeast Queensland, Australia were selected as study sites (Figure 4.1). Creeks with similar attributes, i.e. sediment types, salinity, latitudes, and tidal ranges ( $\sim 1.5$  m) were selected in order to reduce any influence that these factors may have had on the indicators. The creeks are subtropical estuaries with semidiurnal tidal cycles and similar habitat types, comprising mainly mangroves (*Avicennia marina* and *Rhizophora stylosa*). The presence of STPs, septic tanks and agricultural land uses may have contributed to the enriched  $\delta^{15}\text{N}$  values observed in some of the lakes in Chapter 3. While it was impossible to avoid all forms of agricultural land use at the catchment scale, effort was made to reduce the influence of agricultural N sources by avoiding creeks that had catchments with large areas of intensive agriculture. Approximately 50% of the catchments of the two least urbanised creeks are zoned for agricultural and plantation purposes (ABRS 2005). Most of the creeks also had some area ( $\leq 30\%$ ) of their catchment designated for production from natural environments. These areas are mainly used for low density grazing on natural vegetation and may also contribute a small amount of enriched  $\delta^{15}\text{N}$  to the creeks (ABRS 2005). However, the presence of these land uses was well interspersed among the different levels of urbanisation surrounding the creeks. Whilst they may have added to the variability of the indicator responses, their even interspersed amongst the creeks still enabled any net effects of IC and STPs to be detected.

A summary of the catchment land uses and creek characteristics is outlined in Table 4.1. The extent of urbanisation was estimated as the IC in the catchments of the creeks (Table 4.1). This was estimated using the methods described in Chapter 2. To account for differences between the creeks in the catchment to estuary area ratio, IC was multiplied by this ratio to produce an adjusted IC index (AIC) (Table 4.1). Previous studies have used the ratio between catchment area and estuary volume as a measure of sensitivity (Horton & Eichbaum 1991). Unfortunately reliable data for the volume of the creeks was not available at the time of this study. Therefore the area of the estuarine reaches of each creek (creek mouth to upper mangrove limit) was used as a proxy for the volume of each estuary.

Table 4.1. Characteristics of each creek and catchment: land uses, catchment area (CA), creek estuary area (EA), Tidal limit (km upstream from creek mouth), percent impervious cover (IC) and IC, adjusted for creek estuary area (AIC). Creeks marked with an asterisk have treated sewage released into their lower reaches. \*Most (>90%) of the agricultural activity in Bells Creek and Coochin Creek catchments is pine forestry.

Creek	Agriculture /Plantations	Conservation & natural environment	Production from natural environments	Rural residential	Urban residential	Mining & waste	Water environment	CA (ha)	EA (ha)	Tidal Limit (km)	IC (%)	AIC
Bells	57	27	5	0	7	0	4	5389	47.5	5.5	2.3	261.2
Coochin	44	34	15	3	3	0	1	12894	76.2	5.5	2.7	459.1
McCoy's	0	19	8	22	0	0	51	1033	9.4	3.0	4.6	503.9
*Eprapah	6	28	18	33	14	0	1	4334	10.2	3.0	8.1	3427.3
*Hayes Inlet	2	21	30	1	36	1	9	5158	180.7	8.5	13.7	392.3
Saltwater	0	32	17	0	45	0	6	2805	39	10	19	1366.8
*Bulimba	2	19	0	13	65	0	1	11633	67.4	12	26.3	4546.7
*Cabbage Tree	0	12	9	0	73	1	5	4000	21.7	5.5	28.4	5228.3
<b>Source:</b>			(ABRS 2005)					(EPA 2006)		This study		

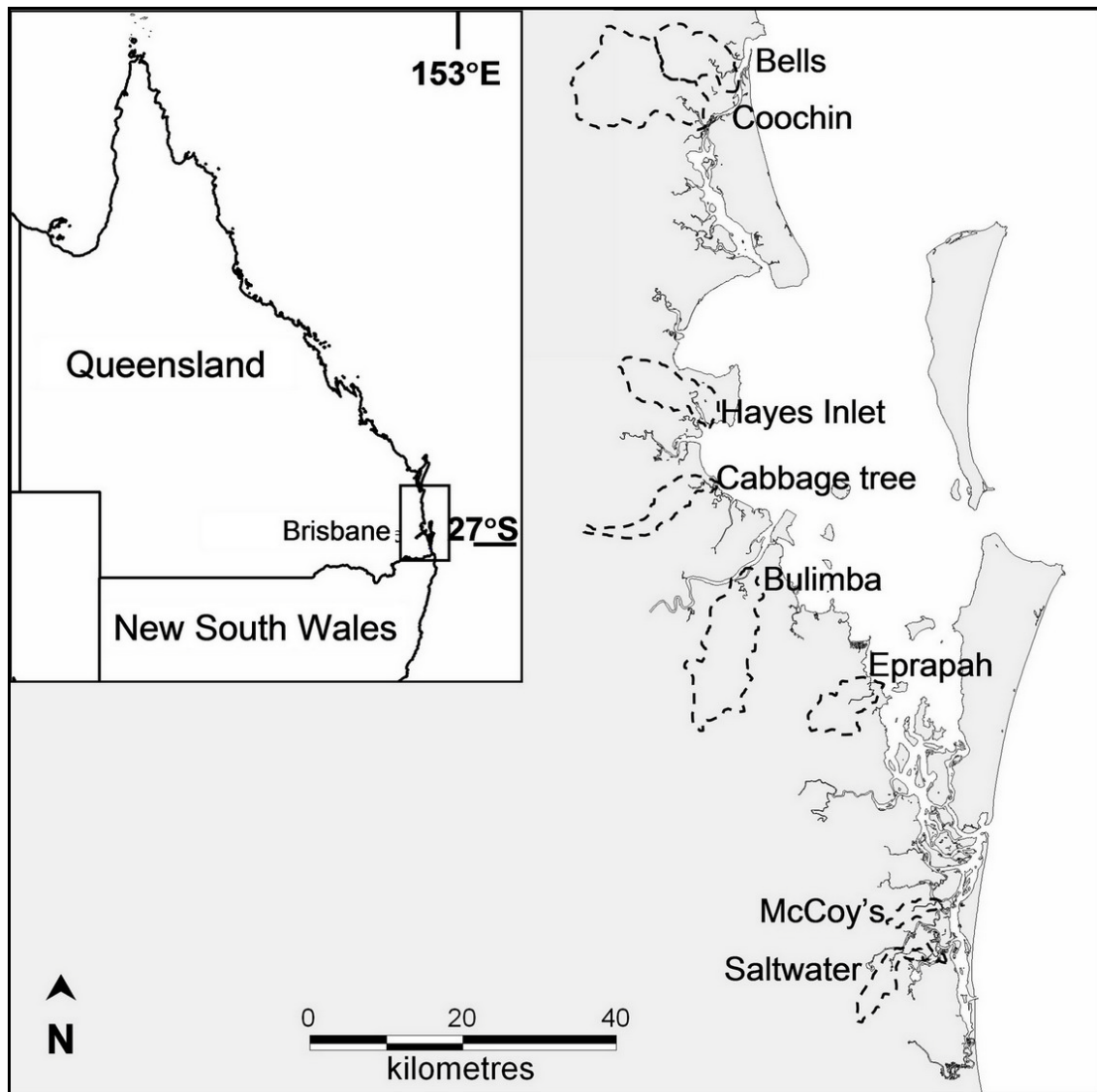


Figure 4.1. Map of southeast Queensland, Australia showing the creeks and catchments (broken lines).

#### 4.3.2 Sampling design and indicator collection

Mangrove leaf, crab and surface sediment samples ( $n = 10$ ) were collected haphazardly from the mid intertidal zone (i.e., within 3 m either side of the mangrove forest / mudflat boundary) of each creek during diurnal low tide periods during 10 – 21 May 2007. Sampling locations were separated by at least 200 m (Figure 4.2). *Australoplax tridentata* was the most common crab species at all 8 creeks, whereas *Parasesarma erythrodactyla* was not common in all of the creeks. Therefore, *A. tridentata* was the only species sampled in this study.

At each creek, 10 mature mangrove leaves (*Avicennia marina*) were collected from each of 10 individual trees and 10 male *Australoplax tridentata*, 8–20 mm



carapace width (CW) were caught and euthanised in an ice slurry. Female crabs were not used because egg production increases variation in the HI (Kyomo 1988). Mangrove and crab samples were stored on ice before freezing at -20°C in the laboratory for later analysis.

The recent acquisition of a portable liquid nitrogen dewar enabled me to collect surface sediment samples for Chl *a* measurement using the contact core technique (Ford & Honeywill 2002). This method is more precise than the coring method used in previous chapters. It enables sample acquisition of the sediment's photic zone (2 mm deep, surface area of 25 cm<sup>2</sup>), where the majority of microalgae are present, providing measures on a scale more relevant to biotic processes than coarser coring techniques (Ford & Honeywill 2002). Cores were carefully wrapped in labelled aluminium foil and frozen in liquid nitrogen and returned to the laboratory for pigment analysis. These samples were used to generate a mean value for each type of indicator from each creek.

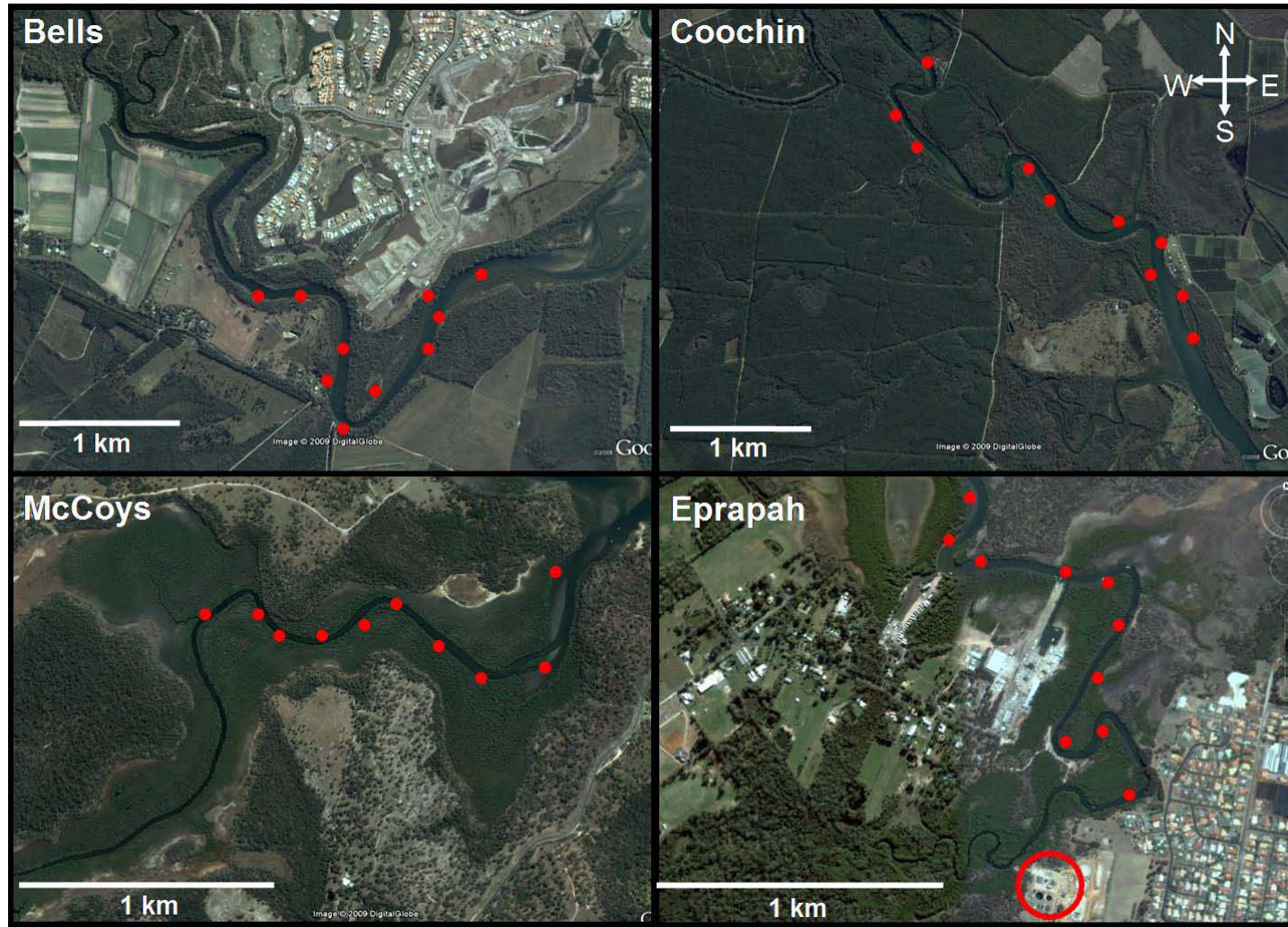


Figure 4.2. Aerial images of each creek, taken from Google Earth (2008). STPs are highlighted with a red circle and red dots indicate the approximate location of the sampling sites in each creek. Considerable development has been occurring within the lower reaches of the Bells Creek catchment since the time of this study.





Figure 4.2. Aerial images of each creek, taken from Google Earth (2008). STPs are highlighted with a red circle and small red dots indicate the approximate location of the sampling sites in each creek.

### **4.3.3 Stable isotope analysis and crab condition (HI)**

Mangrove leaf and crab samples were processed and  $\delta^{15}\text{N}$  and crab HI values were calculated as described in Chapter 2. Precision of the  $\delta^{15}\text{N}$  analysis was better than  $\pm 0.2\%$ , using prawn ( $n = 14$ ) and flour standards ( $n = 12$ ).

### **4.3.4 MPB biomass (Chl *a*)**

MPB biomass was estimated from measurement of the Chl *a* concentration of the surface sediment samples. Pigments were extracted from freeze-dried contact core samples with 90% acetone. Chl *a* concentration ( $\text{mg m}^{-2}$ ) was calculated as described in Chapter 2.

### **4.3.5 Composite biotic condition index (BCI)**

The applicability of each indicator for inclusion in the BCI was determined by the criteria described in Chapter 3.

### **4.3.6 Data analyses**

The indicator samples ( $n = 10$ ) collected from each creek were used to obtain a mean indicator value for each creek. Data were checked for normality and homogeneity of variance, using Komolgorov-Smirnoff and Levene's tests. Where necessary, data were natural log transformed in order to meet these assumptions, as indicated in the results.

All data were checked for linearity with the covariates, AIC and IC, by inspecting scatter plots for each indicator. Where linear relationships were evident between the indicator values and the covariates one-factor ANCOVA were used to test for any effect of STPs and relationships between each covariate, IC and AIC, and the indicators. Where the effect of STPs was non-significant, mean indicator values from all eight creeks were tested for relationships with IC and AIC using regression analyses. If the presence of STPs had any significant effect on the indicators, regression analyses were carried out separately for creeks with and without STPs. Where indicators responded significantly to the presence of STPs but IC and AIC had no significant covariate effect, two-factor nested ANOVA were used to provide a more powerful test for the effect of STPs, as well as to assess any variability in indicator responses between the creeks,  $\text{Cr}(\text{STP})$ .

## 4.4 Results

### 4.4.1 Biotic responses to STPs

Exposure to sewage effluent had a significant effect on mangrove and crab  $\delta^{15}\text{N}$  values, however IC and AIC had no effect on these indicators (Table 4.2).  $\delta^{15}\text{N}$  values were significantly more enriched in mangroves (ANOVA,  $F = 24.34$ , df 1, 6,  $p < 0.01$ , Figure 4.3) and crabs (ANOVA,  $F = 139.77$ , df 1, 6,  $p < 0.01$ , Figure 4.3) in creeks that were exposed to treated sewage. Mangrove  $\delta^{15}\text{N}$  values also varied among creeks (ANOVA,  $F = 32.24$ , df 6, 72,  $p < 0.01$ , Figure 4.3) with  $\delta^{15}\text{N}$  values not showing as much enrichment in Hayes Inlet and Bulimba Creeks as in Eprapah Creek and Cabbage Tree Creek (SNK test, both  $p < 0.05$ ). Among the creeks that were not exposed to sewage effluent, mangrove  $\delta^{15}\text{N}$  values were significantly lower in Bells Creek, the least urbanised of these four creeks (SNK test,  $p < 0.05$ ). Crab  $\delta^{15}\text{N}$  values also varied among creeks (ANOVA,  $F = 7.18$ , df 6, 72,  $p < 0.01$ , Figure 4.3) but only among those which were not exposed to sewage effluent, with  $\delta^{15}\text{N}$  values, again, being significantly lower in Bells Creek, as well as in McCoy's Creek (SNK test, both  $p < 0.05$ ). Exposure to sewage effluent had no significant effect on MPB biomass (Chl *a*) or crab condition (HI) (Table 4.2).

### 4.4.2 MPB biomass and crab condition

Mean sediment Chl *a* concentrations decreased with greater catchment imperviousness (Table 4.2, Linear regression:  $F = 9.36$ , df 1, 6,  $r^2 = 0.61$ ,  $p < 0.05$ , Figure 4.4). Similar to the response observed in Chapter 3, crab HI values displayed an obvious negative response to IC, but this not significant with  $\alpha$  set at 0.05. Again, this may have been due to a lack of power, due to the more varied response in crab HI, compared to Chl *a* and the low number of sites ( $n = 8$ ). Therefore, in order to reduce the risk of a type II error,  $\alpha$  was raised to 0.1 and the relationship between crab HI and IC was significant (Linear regression:  $F = 5.03$ , df 1, 6,  $r^2 = 0.46$ ,  $p < 0.1$ , Figure 4.4). AIC had no effect on Chl *a* or crab HI (Table 4.2).

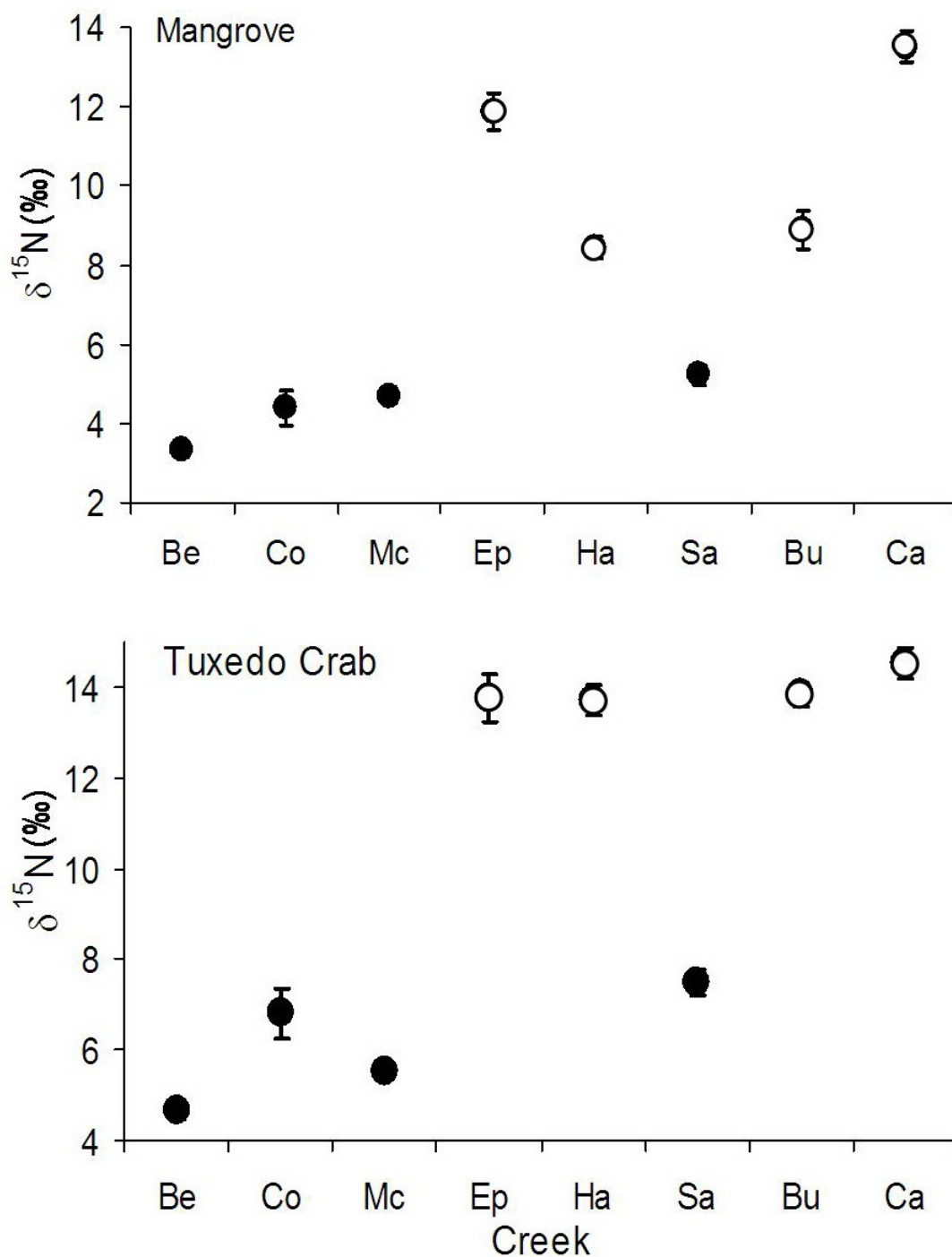


Figure 4.3.  $\delta^{15}\text{N}$  values (mean  $\pm$  SE) of mangrove leaf and crab muscle tissues sampled from the following creeks: Bells (Be), Coochin (Co), McCoy's (Mc), Eprapah (Ep), Hayes Inlet (Ha), Saltwater (Sa), Bulimba (Bu), and Cabbage Tree (Ca). White symbols indicate creeks that were exposed to sewage effluent and black symbols indicate creeks that were not. Samples of each indicator ( $n = 10$ ) were collected from each of the creeks in May 2007.



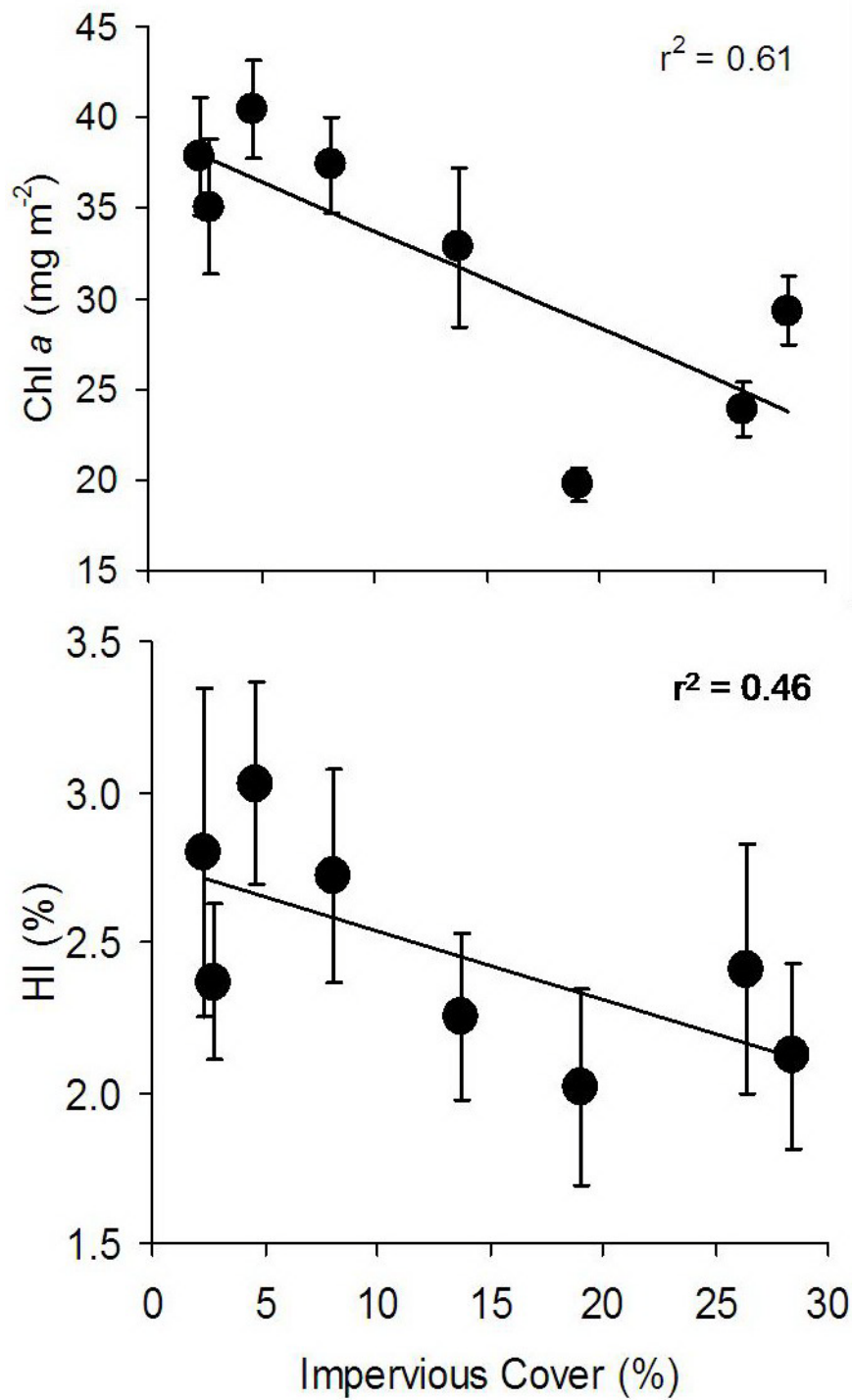


Figure 4.4 a) Chl *a* and b) crab HI (mean  $\pm$  SE) regression relationships ( $p < 0.05$  and  $p < 0.1$ , respectively) with impervious cover (IC). Means are estimated from indicator samples ( $n = 10$ ) collected from eight estuarine creeks around southeast Queensland during May 2007.

Table 4.2. Significance values for two-factor ANCOVA examining the effects of covariates, IC and AIC and the presence of STPs on biotic  $\delta^{15}\text{N}$ , MPB biomass (Chl *a*) and crab hepatosomatic index (HI). Mangrove  $\delta^{15}\text{N}$  values were natural-log transformed before analysis. The homogeneity of variance assumption was satisfied for all analyses, except for those testing the effects of STP and AIC on crab  $\delta^{15}\text{N}$  and STP and IC on mangrove  $\delta^{15}\text{N}$  (Levene's test:  $p < 0.05$ ).

Source	Mangrove $\delta^{15}\text{N}$			Crab $\delta^{15}\text{N}$		Chl <i>a</i>		Crab HI	
	df	<i>F</i>	<i>p</i>	<i>F</i>	<i>p</i>	<i>F</i>	<i>p</i>	<i>F</i>	<i>p</i>
STP	1	12.58	<b>0.016</b>	55.70	<b>0.001</b>	0.17	0.700	0.03	0.860
AIC	1	2.91	0.149	0.72	0.435	1.02	0.360	0.14	0.728
Residual	5								
STP	1	16.57	<b>0.010</b>	92.16	<b>&lt;0.001</b>	2.87	0.151	0.31	0.599
IC	1	0.74	0.428	2.71	0.160	14.49	<b>0.013</b>	4.08	0.100
Residual	5								

#### 4.4.3 Biotic condition (BCI)

Mangrove and crab  $\delta^{15}\text{N}$  values showed no significant response to IC or AIC, therefore these indicators were not included in the BCI. Both Chl *a* and crab HI displayed negative responses to increased IC (Linear regression:  $r^2 = 0.61$ ,  $df = 1, 3$ ,  $p < 0.05$  and  $r^2 = 0.46$ ,  $df = 1, 3$ ,  $p < 0.01$ , respectively). Principal component analysis showed that both indicators made similar contributions to the variance of their combined data set and were positively associated with the first principal component (PC1), which explained 90% of the variance amongst these indicators (Table 4.3). Based on their similar responses to IC, contribution to the PC1, and correlation with each other (Pearson's correlation:  $r = 0.80$ ,  $p < 0.05$ ), Chl *a*, and crab HI were combined into the final BCI. The combined response of these indicators displayed a significant negative relationship with IC (Linear regression:  $r^2 = 0.61$ ,  $p < 0.05$ ,  $df = 1, 3$ , Figure 4.5), but showed no relationship with AIC (Linear regression:  $p > 0.05$ ).

Table 4.3. Coefficients and accountable variance of the first principal component (PC1) based on individual biotic indicators.

Indicator	PC1
Chl <i>a</i>	0.53
<i>Australoplax tridentata</i> HI	0.53
% variance accounted for	90.36



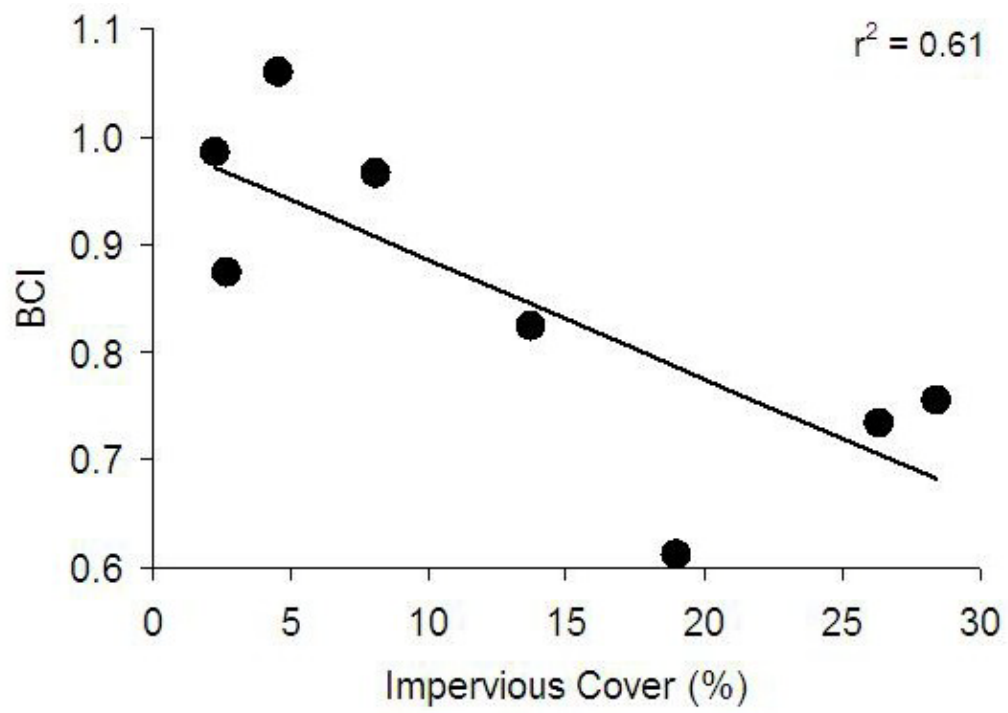


Figure 4.5. Regression relationship ( $p < 0.05$ ) between the biotic condition index (BCI) and IC of the eight creeks.

## 4.5 Discussion

### 4.5.1 $\delta^{15}\text{N}$ , MPB biomass, and crab condition in creeks exposed to sewage discharge

The significant enrichment of mangrove and crab  $\delta^{15}\text{N}$  values in creeks exposed to sewage effluent supports the model that biota would show a greater assimilation of isotopically enriched urban N sources in creeks exposed to STP discharge. This result matches the pattern of enrichment of  $\delta^{15}\text{N}$  in estuarine biota exposed to sewage N in previous studies (e.g., McClelland et al. 1997, Costanzo et al. 2001, Savage 2005, Schlacher et al. 2007). Although an assimilation of sewage N by estuarine biota was evident, results from this study indicate that, so far, this is not having an effect on MPB biomass or crab condition.

The creeks exposed to sewage discharge also had some degree of urbanisation within their catchments (Table 4.1). Therefore, it is possible that other pollutants delivered in urban runoff may have dampened or offset any effect that sewage N had on biota. For example, exposure to increased sediment loads, metals or herbicides may have reduced MPB production and also led to reductions in crab condition (Moreno-Garrido et al. 2003, Elumalai et al. 2005, Ma et al. 2006), potentially canceling out any increase in these variables linked with N inputs from sewage. The lack of response in MPB biomass may also explain why crab condition showed no response to the assimilation of sewage N. *Australoplax tridentata* are thought to derive most of their nutrition from MPB; assuming that the abundance of this species remains constant and that competition for food does not change, its response to any form of urban disturbance is likely to be tightly coupled with those of MPB (Rodelli et al. 1984, France 1998, Oakes 2006).

Although MPB biomass and crab condition showed no response to the assimilation of N from urban sewage, it is possible that the release of treated sewage may affect biota in other ways. Other pollutants in treated sewage, such as ammonia, metals, and synthetic steroids can lead to endocrine disruption and disease in estuarine biota (Depledge & Billingham 1999, Schlacher et al. 2007). A recent study examining estuarine fish responses to the release of tertiary-treated sewage revealed a significantly higher occurrence of tissue pathologies in bottom-feeding mullet (*Valamugil georgii*) exposed to sewage effluent (Schlacher et al. 2007). This coincided with significantly enriched  $\delta^{15}\text{N}$  values in the same species, indicating an assimilation of sewage N from

the benthic habitats in these estuaries. Therefore, the enriched  $\delta^{15}\text{N}$  values of crabs in creeks with STPs may also indicate the presence of other disease causing pollutants in these estuaries.

The continuing urban growth of southeast Queensland is predicted to lead to increases of up to 50% in the release of sewage N into estuaries. The current draft southeast Queensland water strategy proposes the reuse of treated effluent for potable uses (QWC 2008). While this may reduce the volume of effluents released into the region's estuaries, the total nutrient loads (i.e. the product of nutrient concentration and discharge volume) are likely to continue increasing due to population growth in the region. Furthermore, the additional treatment of wastewater for potable purposes will lead to the release of greater concentrates of nutrients and other pollutants, such as metals and endocrine disrupters. Therefore, monitoring of estuarine responses to the release of effluent should be continued so that any impacts can be detected and managed early, before dramatic ecological changes occur.

#### **4.5.2 Assimilation of $\delta^{15}\text{N}$ from urban runoff by mangrove biota and increased imperviousness**

Mangrove and crab  $\delta^{15}\text{N}$  values showed no significant response to IC indicating that urban runoff was not a predominant source of isotopically enriched N in the creeks. Although the assimilation of enriched  $\delta^{15}\text{N}$  was more pronounced in creeks exposed to sewage effluent, the  $\delta^{15}\text{N}$  values of biota in some of the creeks without STPs appeared enriched compared to those reported in relatively pristine environments. For example, the leaves of *Avicennia marina* on Moreton Island have a  $\delta^{15}\text{N}$  of  $\sim 1.7\text{‰}$  (Costanzo et al. 2001). Whereas, *A. marina* leaves collected from three of the creeks which were not exposed to sewage had mean values  $> 4\text{‰}$ . In Chapter 3, the  $\delta^{15}\text{N}$  of *Australoplax tridentata* collected from Lake Weyba was  $\sim 4\text{‰}$ , however, the  $\delta^{15}\text{N}$  values of this species were  $> 6\text{‰}$  in two of the creeks without STPs in this chapter. Although this study did not identify a relationship between  $\delta^{15}\text{N}$  and IC, the enrichment of  $\delta^{15}\text{N}$  in creeks without STPs may have been linked with some assimilation of enriched  $\delta^{15}\text{N}$  in urban runoff, particularly in the more urbanised creeks. Enriched macroalgae  $\delta^{15}\text{N}$  values reported in estuarine habitats exposed to large volumes of urban runoff, indicate that urban runoff can be a predominant source of enriched  $\delta^{15}\text{N}$  in estuaries (Dillon & Chanton 2008). The contribution of enriched  $\delta^{15}\text{N}$  from urban runoff is likely to depend upon rainfall. This appeared to be the case in Chapter 2, when biota around influx sites

showed a greater assimilation of enriched  $\delta^{15}\text{N}$  following rainfall. The weather during the time of this study was predominantly dry, with little or no rain. Further assessment of the relationship between IC and  $\delta^{15}\text{N}$  enrichment in mangrove biota in a larger number of creeks without STPs over a timescale which incorporates rainfall and urban runoff events would aid our understanding of urban runoff contributions to N in estuaries.

#### **4.5.3 MPB biomass and crab condition form a composite index of biotic condition**

MPB biomass and crab condition both showed negative responses to increased urbanisation without IC being adjusted for differences in the catchment to estuary area ratio between the creeks. The combination of these two variables into the BCI, showed a negative relationship with IC. Coincidentally, this was similar to the relationship between IC and Chl *a* (both  $r^2 = 0.61$ ). Together with the strong correlation between the responses of MPB biomass and crab condition, this may imply that MPB biomass alone could be used as a surrogate indicator of BCI. However, the combination of MPB biomass with other biotic variables provides a broader insight of the multi-trophic responses to urbanisation and, therefore, a better indication of the ecosystem level of response. The tight coupling between the responses of MPB biomass and crab condition is likely to reflect a dependence of *Australoplax tridentata* upon MPB as a food source and therefore, may indicate a cascade of urban impact between estuarine primary producers and consumers (Guest et al. 2004, Oakes 2006). Arguably, if the response of MPB biomass to urbanisation reflects the responses of other biotic measures, it could be used individually as an indicator of estuarine health. However, before the responses of MPB are extrapolated to represent ecosystem responses to human impacts, relationships between MPB biomass and the responses of other ecosystem health measures must be established.

The decline in BCI indicates that urban pollutants which inhibit MPB production and consumer condition have a greater impact on mangrove biota than nutrients, which at slight elevations, would be expected to subsidise primary productivity and consumer condition (Odum et al. 1979). Alternatively, urban N inputs (from sewage and runoff) may have also contributed to the decline in biotic condition by stimulating phytoplankton growth, which in turn may have reduced the productivity of submerged MPB by increasing the turbidity of the water column (e.g., Meyercordt &

Meyer-Reil 1999). Nutrient inputs may have also triggered eutrophication and bottom water hypoxia, which would have also negatively affected estuarine biota (Diaz & Rosenberg 1995, Savage et al. 2002). However, the MPB in this study was collected from a shallow intertidal zone, submersed by only ~30cm of water during high tide and emmersed for ~ 12 hour during semi-diurnal low tide. Therefore, bottom water hypoxia and turbidity were unlikely to have a major influence on these indicators. Moreover, exposure to sewage  $\delta^{15}\text{N}$  had no effect on MPB biomass and crab condition, indicating other pollutants delivered in urban runoff, contributed to the decline in biotic condition. The results of this study and of Chapter 5 indicate that pollutants conveyed in urban runoff such as sediments, metals, petrochemicals, and herbicides are the predominant stressors to biota in urbanised estuaries.

#### **4.5.4 System specific characteristics influence the effect of IC on mangrove biota**

Impervious cover provided a good indication of the BCI, without being adjusted for differences in sensitivity between the creeks. This differs to the relationship shown in Chapter 3, where biotic condition showed a decrease with greater IC only after IC was adjusted for differences in the catchment to lake area ratio. Previous studies in estuarine creeks have also detected relationships between IC and biological responses, without having to account for differences in this ratio (e.g., Sanger et al. 1999a, Mallin et al. 2000, Holland et al. 2004). The size of the creeks in relation to their catchments may have been non-significant in this relationship due to the typically shorter residence time of catchment flows in creeks compared to lakes (Soballe & Kimmel 1987). This would provide less time for urban pollutants to accumulate within the creeks – therefore in creeks, faster flow rates are likely contribute more to the dilution of pollutants. Whilst, in slower flowing lakes, size is likely to play a more important role in the dilution of accumulated pollutants (William 1986, Soballe & Kimmel 1987).

The linear relationship between the biotic condition of the creeks and IC differed to the power shaped relationship observed in the estuarine lakes in Chapter 3. This may simply have been due to the low replication of sites, particularly in Chapter 3. In other words, the inclusion of additional data points on the regression in Chapter 3 may actually reveal a linear relationship between AIC and biotic condition in the lakes. However, physical differences between the lakes and creeks may also help to explain the difference in the shape of the relationship. For example, a greater accumulation of

pollutants in lakes due to longer water residence times, may have contributed to the abrupt decline in biotic condition (Soballe & Kimmel 1987). The extent of direct drainage connectivity between IC and receiving water ways may have also influence the shape of the relationship (Walsh 2000). Overall, the decline in biotic condition supports the hypothesis that increased catchment imperviousness would contribute to reductions in MPB biomass and crab condition.

#### **4.5.5 Conclusion**

This study supports the model that greater catchment imperviousness contributes to reductions in MPB biomass and crab condition in urbanised estuarine creeks. The negative relationship between IC and the biotic condition index indicates that pollutants in urban runoff, conveyed into the creeks via impervious surfaces contribute to a decline in the condition of biota. Enriched  $\delta^{15}\text{N}$  values in mangrove and crabs from creeks exposed to sewage effluent indicated an assimilation of sewage N into the estuarine food web. This, however, had no effect on the BCI, potentially because pollutants in urban runoff were offsetting any effects of sewage inputs.

As strategies are implemented to mitigate urban runoff pollutants, their counteractive effects on sewage N are likely to reduce. This may lead to eutrophication problems in estuaries exposed to sewage discharge. An understanding of the interactive effects that nutrients and other stormwater pollutants have on estuarine biota is vital for the effective management of coastal urbanisation and the preservation of estuaries.

In this study, the relationship between IC and biotic condition differed to that between AIC and biotic condition in Chapter 3 indicating that physical differences between estuarine systems need to be considered when applying IC to estimate estuarine responses to surrounding urbanisation.



## **Chapter 5. Biotic responses to two urban stressors: sedimentation versus nitrogen enrichment**

### **5.1 Abstract**

Urbanisation increases impervious surfaces that channel stormwater into coastal estuaries. Urban runoff delivers nutrients essential for primary production, but also transports sediments that can increase turbidity and reduce primary production in estuaries. In order to manage these pollutants effectively, we need to understand how estuarine biota respond to their potential antagonistic effects. In the previous chapters reductions in MPB (microphytobenthos) biomass and crab condition both in estuaries which were exposed to sewage inputs and in estuaries which weren't indicates that urban pollutants such as sediments may have a predominant effect on biota over nutrients in these systems.

Using 20 mesocosms to imitate natural conditions, I examined the responses of mangrove biota to the interactive effects of simulated urban runoff additions of nitrogen (N) and suspended sediments. I tested the model that the biomass of MPB (measured as chlorophyll *a* concentration, Chl *a*) would respond positively to N additions, negatively to sediment additions, and also negatively to a combination of N + sediment additions. I predicted that these responses would cascade to consumers, being reflected in the condition of the tuxedo crab, *Australoplax tridentata*, which obtains much of its energy from MPB.

MPB biomass showed no response to N additions, but decreased significantly in response to sediment and sediment + N additions, thus supporting the model that sediment inputs have a greater effect on MPB production than nutrient inputs. This may have been because the ambient turbidity created by the mangrove mud in the mesocosms made MPB production light limited rather than N limited. The responses in MPB biomass, however, were not reflected in crab condition. Crab condition showed no response to any of the treatments, possibly because the crabs exposed to sediment and sediment + nutrient additions were able to adapt to the reduction in MPB by consuming an increased proportion of dead MPB, which according to phaeopigment levels seemed to occur in the sediment treatments. The long-term effects of sediment inputs are, however, likely to reduce the total amount of MPB, as less dead algae is produced from a diminishing stock of living MPB. Over time, crab condition may reflect this eventual decline in total MPB, displaying a similar cascade of response to



that indicated in Chapters 3 and 4. Results from this chapter indicate that sediment inputs have a greater effect than nutrient inputs in turbid, mangrove environments, where light is the most likely factor limiting primary production.

## 5.2 Introduction

Urbanisation of coastal areas is degrading the health of estuaries around the world (Vitousek et al. 1997). Urbanisation along the coast of southeast Queensland is increasing rapidly with the regional human population projected to increase from 2.9 to ~ 4 million by 2026 (OUM 2004, DIP 2008). The region has a diverse array of estuarine environments, including coastal embayments, rivers, lagoons, shallow lake systems, and tidal creeks. These ecosystems provide vital refuge and food to many fish, invertebrate, bird, reptile, and marine mammal species (Kneib 1997, Lee 1999, Nagelkerken et al. 2008).

Due to the rapid urban growth in southeast Queensland, nutrient and sediment loads entering estuaries in urban runoff are expected to increase by more than 50% during the next 20 years. Nutrients from tertiary-treated sewage, are expected to increase by up to 50% (EPA 2007). In order to meet the growing population's demand for potable water, future strategies include the additional treatment of sewage so that it may be re-used for potable purposes (QWC 2008). This is likely to increase the concentration of nutrients and other pollutants discharged into coastal areas as sewage effluent. In order to manage urban pollutants effectively, we need to understand how they interact with each other, as well as how they affect estuarine biota.

Urbanisation of the region's coastal areas is already impacting the water quality of estuaries (EPA 2007). The previous two chapters have also indicated a decline in the biotic condition of estuaries around the region as urbanisation (measured as IC) increases. The  $\delta^{15}\text{N}$  values of crabs and mangroves also indicated that sewage treatment plants (STPs) and septic tank systems contribute substantially to the presence of urban nitrogen (N) in these estuaries. The assimilation of urban N by mangrove biota, however, was not connected to the decline in biotic health. Instead, biotic health was associated with the IC surrounding the estuaries, indicating that pollutants in urban runoff have a greater impact on the estuaries than N derived from sewage. The decline in biotic health indicates that urban runoff delivers pollutants such as sediments and metals, which inhibit benthic primary production and crab condition (Schueler 1987, Moreno-Garrido et al. 2003, Elumalai et al. 2005). These pollutants seem to dominate the effects of urban N, which typically stimulates primary production in estuarine environments (Mallin et al. 2004, Kemp et al. 2005, Lever & Valiela 2005). Few studies have examined the effects of N additions on MPB (microphytobenthos)

production in the presence of other stressors. Although, recently Stutes et al. (2006) documented that in a turbid, nutrient-rich estuary, increased shading had a greater effect on MPB production than nutrient enrichment.

Benthic microalgae play an important role in shallow estuarine ecosystems as oxygen producers, carbon fixers and food sources for consumers (MacIntyre et al. 1996, Lee 2000, Bouillon et al. 2008). As urbanisation in coastal areas continues to grow, there is a need to understand how urban N inputs interact with other urban stressors in determining the overall response in MPB production and how these responses influence estuarine biota that rely upon this resource (Cloern 2001).

This chapter examines the interactive effects of N and sediment additions on the responses of MPB biomass and consumer (crab) condition. Mesocosms of mangrove mud were exposed to eight pulses of N and sediment additions over one month, simulating urban runoff containing these pollutants. I tested the model that the effects of sediment would be greater than the effects of N on biota. This would be demonstrated by MPB biomass and crab condition responding to interactive N and sediment addition effects. I predicted that N addition would stimulate benthic primary production, causing an increase in MPB biomass, that sediment additions would reduce MPB biomass due to increased turbidity, and that combined N and sediment additions would also decrease MPB biomass, due to turbidity have a stronger effect on MPB production than N addition. I expected these effects to cascade from the MPB to one of its consumers, the tuxedo crab, *Australoplax tridentata*, (France 1998, Guest et al. 2004, Doi et al. 2005). This would be demonstrated by an increase in the condition (hepatosomatic index, HI) of crabs exposed to N inputs, due to the stimulation of MPB biomass by the N additions, a decrease in the condition of crabs exposed to sediment inputs due to reductions in MPB biomass, and a reduction in the condition of crabs exposed to combined N and sediment additions, due to the stronger effect of sediments on MPB biomass.

The following hypotheses were tested:

(1) Chl *a* would be greater in mesocosms with N additions than in mesocosms without N additions, and depleted in mesocosms with both sediment, and sediment + N additions compared to mesocosms without sediment additions, and;

(2) body condition (HI) of the ocyopodid crab *Australoplax tridentata* (tuxedo crab) would be greater in mesocosms with N additions than in mesocosms without N

additions, and depleted in mesocosms with both sediment, and sediment + N additions compared to mesocosms without sediment additions.

## 5.3 Methods

### 5.3.1 Mangrove mud mesocosms

Intact blocks of intertidal mangrove mud (40 × 40 cm wide, 10 cm deep) were collected with associated microalgae and macrofauna from Lake Coombabah (27°55' S 153°21' E) during diurnal low tides on 12 Sept 2007. The blocks of mud were placed into black PVC containers (40 × 40 cm wide, 35 cm deep), returned to the laboratory and maintained at 21°C. Macrofauna > 5 mm, which were comprised mainly of other crab species, including *Heloecius cordiformis*, *Uca longidigita*, *Helograpsus haswellianus*, and *Macrophthalmus setosus*, were removed from the mesocosms by hand, and by placing a pitfall trap (5 cm diameter) into the centre of each mesocosm. During this process, care was taken to minimise disturbance to the sediment. Animals were removed from the pitfall traps each day, until the end of the defaunation period (two weeks), when lids were screwed onto the empty pitfall traps. The traps were left in the mesocosms to avoid unnecessary disturbance to the mud associated with removing the traps.

The mesocosms ( $n = 20$ ) were subjected to simulated semi-diurnal tidal cycles using filtered seawater collected from the Gold Coast Seaway (27°56' S 153°25'E) during incoming tides. This was diluted using deionised water to achieve salinity similar to that of Lake Coombabah ( $26.7 \pm 2.2\%$  (SE)). Each mesocosm was allocated 10 L of tidal water which was stored next to each mesocosm in PVC plastic reservoirs with air bubblers to circulate the water (Figure 5.1). Water pumps, set to an automatic timer switch, pumped tidal water into the mesocosms twice each day for a period of 2 – 3 hours. A tidal outflow pipe (1 cm diameter) maintained the high tide water level at a depth of ~ 10 cm above sediment surface (Figure 5.1). This achieved a depth and period of immersion within the range of the high tide conditions that occur in the intertidal mangrove forest of Lake Coombabah. At the end of each high tide period, the tidal water was drained back into the reservoirs via a gravity fed outflow pipe (3 mm diameter) located at the sediment surface. The tidal water took ~30 minutes to drain from the mesocosms. Although air bubblers were used to circulate the water, tidal water was changed every three days to reduce any build up of organic material; this

maintained DO levels above 80% saturation. Fluorescent lights connected to an automatic timer switch were used to produce a 12 hour light: 12 hour dark photo-regime throughout the experiment. The fluorescent tubes produced a photon flux of  $25.2 \pm 1.2 \mu\text{mol m}^{-2} \text{s}^{-1}$  (SE) 20 cm above the mud surface. This was similar to that recorded at Lake Coombabah in the mangrove forest around midday on a fine day ( $29.6 \pm 15.2 \mu\text{mol m}^{-2} \text{s}^{-1}$  (SE)).

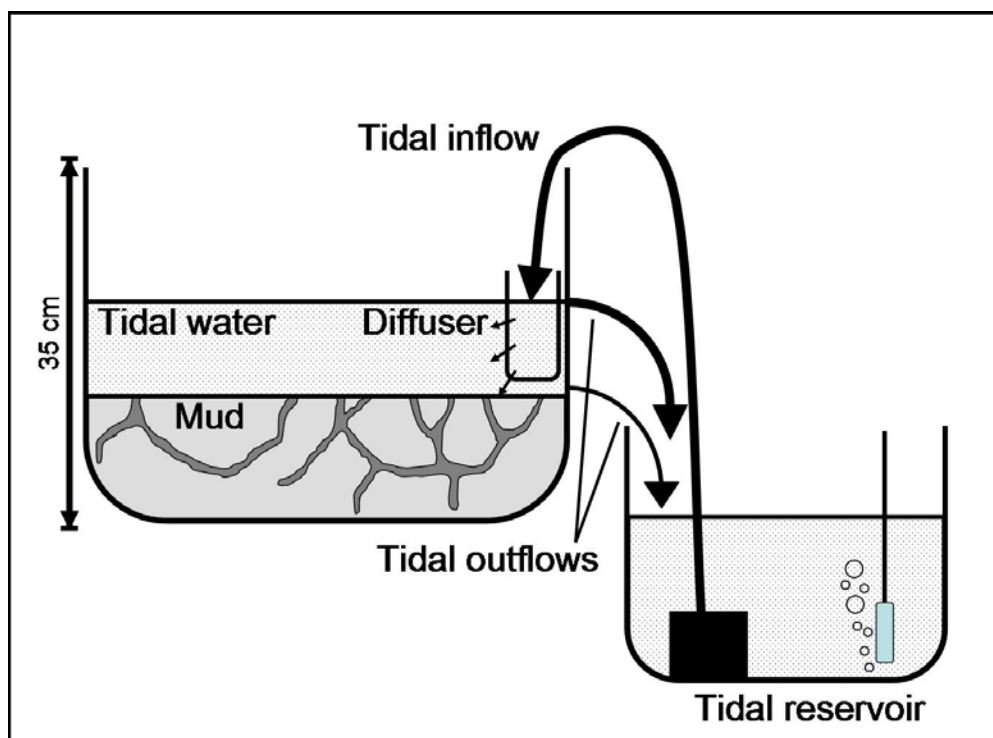


Figure 5.1. Schematic diagram of the mesocosm and tidal reservoir, with water pump and air bubbler. Tidal water was pumped twice for three hours every 24 hours into mesocosms via a plastic diffuser. A tidal outflow pipe (1 cm diameter), 10 cm above the sediment surface maintained water levels at a depth of 10 cm during high tide. Another outflow pipe (3 mm diameter) located at the sediment surface drained tidal water from the mesocosms to simulate ebb tides.

### 5.3.2 Crab stocking density: pilot study

In the main experiment, the replicate values of crab HI were based on a mean HI value from each mesocosm (i.e. 1 mesocosm = 1 replicate). This was done to reduce the variance of the mean crab HI value for each treatment and therefore, increase the power to detect any response to the sediment and N additions. However, I did not want to risk overstocking the crabs, therefore I did a power analysis to determine the minimum number of crabs required to provide at least a 60 – 80% chance of detecting a response. I then did a pilot study to test that the mesocosms could sustain this number

of crabs. The power analysis was done using the crab HI data from Chapters 3 and 4, and crab HI values reported in the literature to estimate the likely effect size of the N and sediment additions, as well as the variance of crab condition (Kennish 1997, Moreno-Garrido et al. 2003). The results indicated that a stocking density of five crabs per mesocosm would provide 65 – 80% power to detect a minimum change of 30% in crab condition.

In addition, a pilot study investigating whether *Australoplax tridentata* occurred at densities similar to this in Lake Coombabah indicated that the crabs occur at a density of  $4.5 \pm 0.6$  individuals per  $30 \times 30$ cm quadrat ( $n = 10$ ). Crab numbers were estimated by counting the number of crabs within each quadrat. Crabs were enticed out of their burrows by rapidly inserting a blunt knife at an angle alongside and then into each burrow. A recovery rate of  $95 \pm 4\%$  has been reported for this method when used on similar ocypodid species (Taylor & Allanson 1993).

In order to check that the mesocosms would be able to sustain five crabs under laboratory conditions, a further pilot study was carried out. This tested the hypothesis that in mesocosms overstocked with crabs, MPB biomass (Chl *a*) would decrease through time, but in mesocosms containing a sustainable number of crabs, MPB biomass would remain stable through time. Twelve mesocosms were stocked with crabs at densities of 0, 1, 3 and 5 crabs ( $n = 3$ ) and sediment samples (one 5.7 cm diameter contact core per mesocosm) were collected every two days over an eight day period from each of the mesocosms (Ford & Honeywill 2002). A two-way repeated measures ANOVA was used to test for the effects of Time (5 levels) and Crab Density (4 levels). Chl *a* showed no main or interactive responses (all  $p > 0.05$ ) to either of these factors, remaining stable throughout the experiment and indicating that each mesocosm would be able to sustain five crabs throughout the main experiment. All crabs used in this pilot study were carefully removed and the mesocosms left to recover from the Chl *a* sampling activities for 10 days.

### **5.3.3 Collection of crabs**

On the 22 Oct 2007, 120 male *Australoplax tridentata* crabs (8–20 mm carapace width) were collected from the intertidal mangrove flats of Lake Coombabah during diurnal low tide. Female crabs were not used because egg production increases variation in HI (Kyomo 1988). The crabs were hand collected from a site located well away from any urban influx sources ( $27^{\circ}54' \text{ S } 153^{\circ}20'$ , and see Chapter 2). This was

done in order to avoid using crabs which may have developed a tolerance to urban runoff. The crabs were stored in plastic containers of mud from the collection site, and were returned to the laboratory within two hours of collection. In the laboratory, five crabs were placed haphazardly into each mesocosm and left to acclimatise to laboratory conditions for two weeks. The remaining 20 spare crabs were placed into four mesocosms (five crabs per mesocosm) and subjected to the same conditions as the experimental crabs. These crabs were used to replace any crabs in the main experiment that died or escaped.

### 5.3.4 Experimental design

The main experiment was carried out 10 Nov – 14 Dec 2007. Nitrogen (N) and sediment additions were randomly assigned to each of the 20 mesocosms ( $n = 5$  mesocosms per treatment) (Figure 5.2). Turbidity and total N loads previously reported in urban runoff and in receiving waterways were used to infer the appropriate sediment and N concentrations to add to the mesocosms (e.g. Waltham 2002, CWP 2003, Barry et al. 2004). Sediment and N were added to the mesocosms during simulated diurnal high tides every 4 days. The tidal water in the mesocosms' reservoirs was changed three days after each addition; this simulated an urban runoff residence time of three days with gradual declines in turbidity as sediments settled within the mesocosms (Table 5.1). N levels within the water column remained elevated for the three days after each addition (Table 5.2).

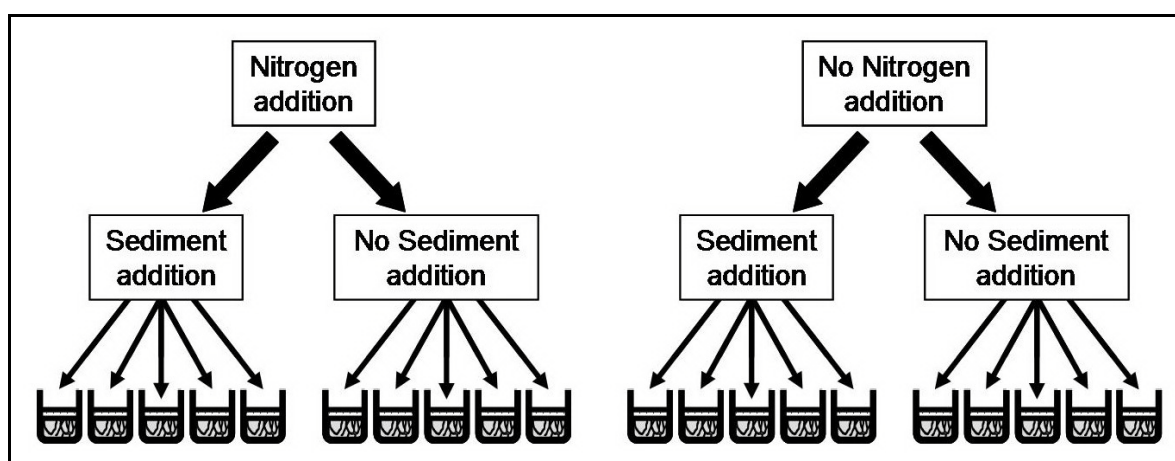


Figure 5.2. Experimental design showing a two-factorial design of nitrogen and sediment additions. Nitrogen and sediment additions were haphazardly allocated to 20 mesocosms. The mean Chl *a* ( $n = 3$ ) and crab HI ( $n = 5$ ) values for each mesocosm were used as replicates ( $n = 5$ ) for each level of N and sediment addition.

#### **5.3.4.1 Sediment additions**

Mud collected from the sample site was used for sediment additions, after being oven dried at 60°C, homogenised and sieved. The fraction of sediments < 62.5 µm diameter (silts and clays) were added to deionised water to attain a sediment concentration of 3 g L<sup>-1</sup> (Wentworth 1922). One hundred milligrams of this was poured slowly into the appropriate mesocosms during the simulated diurnal high tides. This resulted in an initial mean turbidity of 256 ± 23 ntu (SE), which reduced to 125 ± 10 ntu on the second and third days of the sediment additions (Table 5.1). Turbidity in the control and N treatments was 51 ± 7 ntu and 47 ± 4 ntu, respectively.

#### **5.3.4.2 Nitrogen additions**

Nitrate and urea were added to N and sediment + N treatments as a pulse during semi-diurnal high tides using a pipette to transfer 10 mL from a stock solution of Total Nitrogen (TN, 3.2 g L<sup>-1</sup>) containing 1.3 g of Ca(NO<sub>3</sub>)<sub>2</sub>·4H<sub>2</sub>O and 1.9 g of (NH<sub>2</sub>)<sub>2</sub>CO. This resulted in an enrichment of 3.2 mg L<sup>-1</sup> of TN in the mesocosms with an inorganic N: organic N ratio of 1:1.5, similar to the concentration and composition of TN previously reported in urban runoff (CWP 2003, Barry et al. 2004, Taylor et al. 2005). This is a considerably higher concentration than the guideline value for TN of 0.45 mg L<sup>-1</sup> for estuaries in southeast Queensland (EPA 2006). In order to verify that these additions increased TN to the desired level in the mesocosms, the level of Total Filterable Nitrogen (TFN) was measured. Water samples were collected in clean plastic vials, filtered (Whatman GD/X 25 mm, 1 µm glass fibre filter) and stored frozen at -20°C until analysis. Prior to nutrient analysis, all laboratory equipment was cleaned by soaking in 10% HCl and rinsing with Milli-Q deionised water. All reagents used for the measurement of TFN were of analytical grade. TFN levels were measured using the peroxodisulfate oxidation method for TFN in water (Ebina et al. 1983). This was done using an EasyChem Plus automatic sequential colorimetric analyser (Analytical Technologies, Rome, Italy). Detection limits for TFN were 0.04 mg L<sup>-1</sup> and the recovery rate was 95.6%. TFN levels of 3.0 mg L<sup>-1</sup> ± 0.4 were maintained in the water column for three days in the N and N + sediment treatments (Table 5.2).



Table 5.1. Turbidity in mesocosms of the four treatments. S = sediment, N = nitrogen. Mean ( $\pm$ SE,  $n = 3$ ) values (ntu) in over three days. Values which differ significantly from values in other treatments are underlined and values which differ between days are shown in bold ( $\alpha = 0.05$ ).

Day	Control	+ N	S + N	+S
1	40.8 $\pm$ 11.4	34.1 $\pm$ 4.3	<b><u>247.7 <math>\pm</math> 39.9</u></b>	<b><u>263.3 <math>\pm</math> 31.5</u></b>
2	54.2 $\pm$ 8.2	54.6 $\pm$ 6.2	<u>123.5 <math>\pm</math> 21.9</u>	<u>119.1 <math>\pm</math> 28.7</u>
3	58.5 $\pm$ 16.9	53.4 $\pm$ 6.6	<u>131.5 <math>\pm</math> 17.1</u>	<u>124.0 <math>\pm</math> 25.5</u>

Table 5.2. Total filterable nitrogen in the water column of mesocosms of the four treatments. Mean ( $\pm$ SE,  $n = 3$ ) TFN values ( $\text{mg L}^{-1}$ ) over three days. Underlined values are significantly different from those which are not underlined ( $\alpha = 0.05$ ).

Day	Control	+S	+N	S + N
1	0.3 $\pm$ 0.0	0.3 $\pm$ 0.1	<u>3.5 <math>\pm</math> 0.8</u>	<u>2.8 <math>\pm</math> 0.8</u>
2	0.2 $\pm$ 0.0	0.4 $\pm$ 0.1	<u>3.4 <math>\pm</math> 0.4</u>	<u>3.5 <math>\pm</math> 0.3</u>
3	0.1 $\pm$ 0.0	0.4 $\pm$ 0.1	<u>3.1 <math>\pm</math> 0.1</u>	<u>2.1 <math>\pm</math> 0.0</u>

#### 5.3.4.3 Indicator collection

At the end of the experiment, three cores of surface sediment ( $\sim 2$  mm deep, surface area of  $25 \text{ cm}^2$ ), were collected from each mesocosm using the contact core technique (Ford & Honeywill 2002). Cores were wrapped in labelled aluminium foil and stored frozen at  $-20^\circ\text{C}$  until pigment analysis, using methods described in Lorenzen (1967). The three cores were used to generate a mean Chl  $a$  value for each mesocosm. The five crabs were collected from each mesocosm, euthanised in an ice slurry and stored frozen at  $-20^\circ\text{C}$  until measurement of the HI. The five crabs were used to produce a mean HI value for each mesocosm.

#### 5.3.5 Measurement of Chl $a$ , and HI

Sediment and crab samples were prepared and Chl  $a$ , phaeopigment and HI values were measured as detailed in previous chapters. Phaeopigment concentrations were calculated using a modified version of the Lorenzen (1967) equation:

$$\text{Phaeopigments } (\mu\text{g m}^{-2}) = \frac{A \times K (R \times 665_a - 665_i) \times v}{S.A. \times l}$$

$A$  is the absorption coefficient of Chl  $a$  (11.0),  $K$  (2.43) equates the reduction in absorbance after acidification to the initial Chl  $a$  in each sample,  $R$  (1.7) is the maximum ratio of  $665_i : 665_a$  in the absence of phaeopigments,  $665_i$  is the initial absorbance,  $665_a$  is the absorbance after acidification,  $v$  is the volume of acetone used for extraction (ml), S.A. is the surface area of mud sampled ( $\text{m}^2$ ),  $l$  is the path length of the cuvette (cm).

### 5.3.6 Data analysis

The mean Chl  $a$  and crab HI values calculated for each mesocosm were used as replicates for each level of N and sediment treatments (Figure 5.2). Two-way ANOVAs were used to test for any main and interactive effects that N and sediment additions had on Chl  $a$  and crab HI. Data were checked for normality and homogeneity of variance, using Komolgorov-Smirnov and Cochran's tests, respectively. Where necessary, data were natural-log transformed in order to meet these assumptions.

## 5.4 Results

Chl *a* showed a significant response to sediment addition with significantly lower values in mesocosms with sediment additions and in mesocosms with N + sediment added (Figure 5.3, Table 5.3). But did not show any interactive response to N and sediment addition, or any response to N inputs (Table 5.3). Crab HI did not show any significant main or interactive responses to N or sediment additions (Figure 5.3). Although not included in the initial model, it is interesting to note that phaeopigment concentrations indicated an opposite pattern to Chl *a* in response to N and sediment additions, with values appearing higher in mesocosms with sediments added than in the other treatments (Figure 5.3). These responses, however, were not significant (two-way ANOVA: all  $p > 0.05$ ).

Table 5.3. Two-factor ANOVA results examining effects of N and sediment additions on Chl *a*, phaeopigments, and crab HI. All datasets were natural-log transformed before analysis. The homogeneity of variance assumption was satisfied for all indicators. p values shown in bold denote significance where  $\alpha = 0.05$ .

Source	Chl <i>a</i>				Phaeopigments			Crab HI		
	df	MS	<i>F</i>	p	MS	<i>F</i>	p	MS	<i>F</i>	p
N	1	0.00	0.11	0.743	0.03	1.47	0.243	0.00	0.04	0.852
S	1	1.04	31.16	<b>&lt;0.001</b>	0.05	2.53	0.131	0.01	0.05	0.832
N × S	1	0.01	0.15	0.705	0.00	0.01	0.909	0.08	0.64	0.434
Residual	16	0.03						0.12		

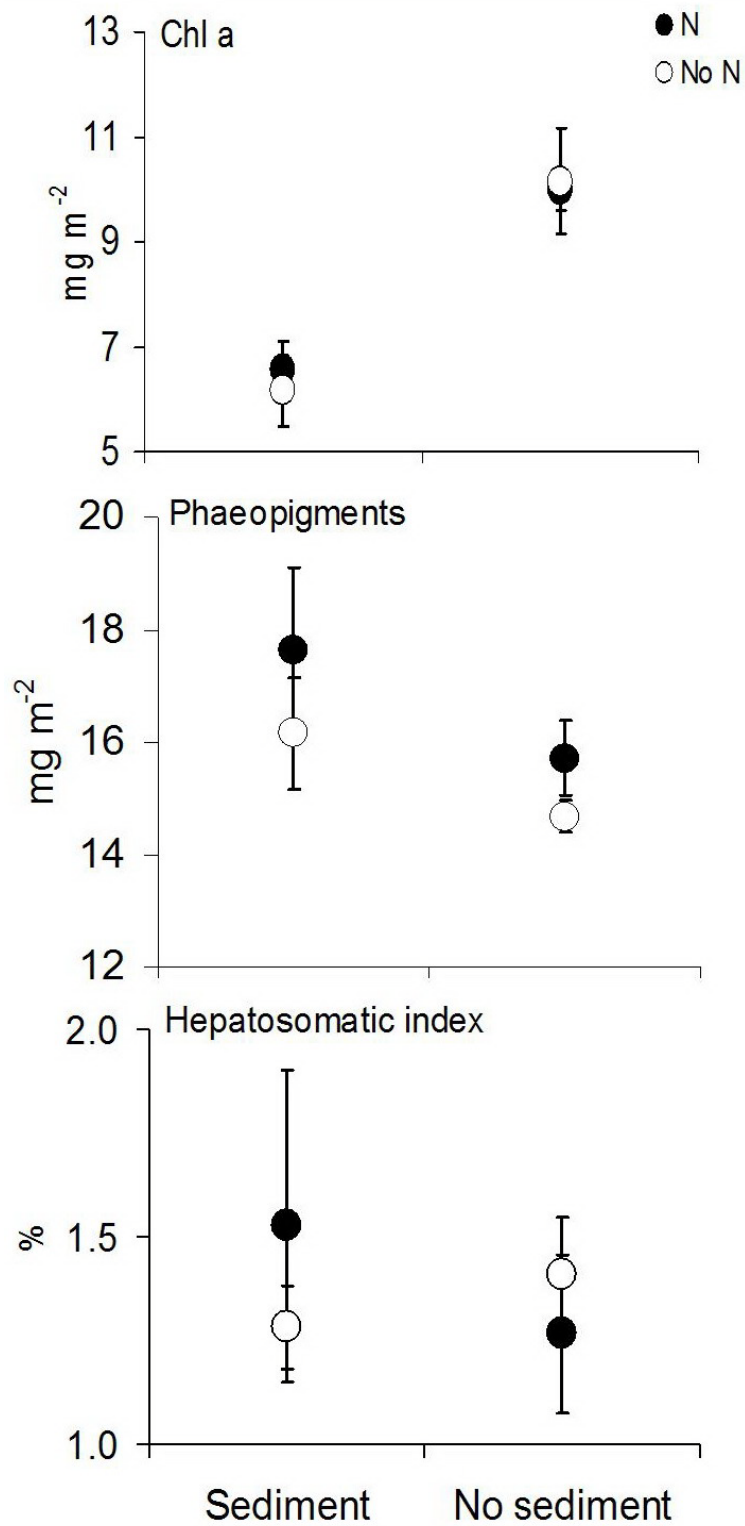


Figure 5.3. Mean ( $\pm$  SE) Chl *a*, phaeopigment concentration and crab HI values in mesocosms with different Sediment and N additions. Sediment Chl *a* was significantly lower in mesocosms with sediment additions ( $\alpha = 0.05$ ).

## 5.5 Discussion

### 5.5.1 MPB biomass

The hypothesis that increased sediment loads reduce MPB biomass was supported by the decreased sediment Chl *a* concentrations in mesocosms with sediment additions. The addition of N, however, had no effect on Chl *a*, irrespective of whether sediment had been added or not. Sediment Chl *a* was also depleted in mesocosms with N + sediment additions, supporting the model that under the experimental conditions, sediment inputs had a predominant effect on MPB biomass over N inputs.

#### 5.5.1.1 *Light limitation under ambient turbid conditions*

Nutrient enrichment has previously been linked with increases in benthic primary production in estuaries (e.g., Cadée 1984, Lever & Valiela 2005). A number of factors may explain the lack of increase in MPB biomass displayed in this study. The N additions may have triggered an increase in the density of phytoplankton in the tidal water of the mesocosms, which may have increased the shading of the submerged MPB, thus counterbalancing the effects of the N additions on MPB biomass. Meyercordt & Meyer-Reil (1999) reported that greater shading due to increased phytoplankton production in a eutrophic estuary had a detrimental effect on MPB primary production. Whilst Chl *a* levels were not measured in the water column in this experiment, turbidity levels were similar in mesocosms with and without N additions (Table 5.1) indicating that increased shading due to greater phytoplankton density was not the reason for the lack of response displayed by MPB biomass to N addition. A simpler explanation for the lack of increase in MPB biomass may have been that the crabs in the N-enriched mesocosms consumed any surplus MPB, dampening any effect that the N additions may have had on MPB biomass. If this were the case, however, one would expect the crabs in the N-enriched mesocosms to have an increased condition index (Kennish 1997). But crab condition showed no significant increase with N additions.

Rather than showing an overall increase in MPB biomass, the species composition of MPB may have changed in response to the addition of N. Increases in the proportion of pollution-tolerant to pollution-sensitive MPB species have previously been linked with increased N inputs (e.g., Underwood et al. 1998). This shift in MPB composition, however, is thought to be largely influenced by a tolerance for high ammonium concentrations (Underwood et al. 1998). This experiment, however, did not

include ammonium in the N additions due to concerns about its toxicity to diatoms at high ( $> 1$  mM) concentrations (Admiraal 1984, Underwood et al. 1998). Therefore, the composition of diatom species is unlikely to have been adversely affected by the N additions. The nitrate and urea additions, however, may have led to an initial increase in MPB with a subsequent increase in detritus, due to the turnover of a greater MPB biomass. This in turn may have increased the ammonium in the mesocosms which had N added. However, phaeopigment concentrations were no greater in mesocosms with N additions, indicating no increase in the amount of dead algae and, therefore, no likely increase in ammonium.

Alternatively, the absence of ammonium from the N additions may explain the lack of response in MPB biomass. Although nitrate is considered the primary source of new production in marine phytoplankton (Underwood et al. 1998, Carter et al. 2005), MPB often demonstrate an assimilation preference for ammonium over nitrate, as incorporation of ammonium requires little change in the oxidation state of N compared to nitrate (Admiraal et al. 1987, Underwood et al. 1998). Therefore, the absence of ammonium may have prevented any significant increase in MPB biomass. Another important element, phosphorus, was also not included in the N additions, and the N:P ratio might have increased to a level that rendered MPB production P-limited (Hillebrand & Sommer 1999). However, (Stutes et al. 2006) also reported no increase in MPB production in response to nutrient additions including ammonium, nitrate and phosphorus. This study was carried out in a turbid, nutrient-rich estuary and the lack of response to nutrient additions was interpreted as MPB productivity being limited by light due to high turbidity levels and nutrient depletion (Stutes et al. 2006).

The mangrove mud used in the mesocosms was collected from Lake Coombabah, a moderately urbanised, turbid, nutrient-rich estuary (Waltham 2002). While all effort was made to collect mud away from urban influx sites, nutrient levels in the mud may have been at a high enough level to prevent them from being limiting to MPB production, particularly under the turbid conditions of the estuary. The tidal water in the mesocosms reached turbidity levels of  $\sim 50$  ntu within one tidal cycle, thus recreating turbid estuarine conditions. Under these simulated conditions, the lack of increase in MPB biomass in response to N additions provides support for the idea that in nutrient-rich, turbid estuaries, MPB production is limited by light more than nutrients. However, because the mud was collected from an urbanised estuary it is not possible to rule out any contribution that urbanisation may have made to this light limited state.

#### 5.5.1.2 *Nutrient depletion may make MPB more responsive to sediment additions*

The decrease in MPB biomass in response to sediment addition adds support to the model proposed in previous chapters that sediment loads in urban runoff contribute to the decline in MPB biomass in estuaries around southeast Queensland. The mechanisms by which this occurs remain unclear. Previous studies have indicated that sedimentation may lead to increases in certain bioturbating macrofauna. Anderson et al. (2004) documented increased numbers of *Helice* sp. and *Macrophthalmus* sp. crabs in 'high'-deposition areas of an urbanised estuary. This model and its mechanisms have yet to be tested. The increased deposition of fine, silty sediments may, however, provide a softer refuge for new recruits to burrow into, improving their chances of survival against predation. If increases in the density of bioturbators such as crabs are associated with sedimentation, this is likely to contribute to declines in MPB biomass due to increased grazing and bioturbation pressures (e.g., Taylor & Allanson 1993, Lever & Valiela 2005, Armitage & Fong 2006). In this experiment, however, macrofauna density was standardised, enabling the effects of sedimentation to be interpreted separately from these potential top down effects. Therefore, this study demonstrates that sedimentation leads to decreases in MPB biomass, independent of any associated top down effects. However, when present, changes in macrofauna composition may behave synergistically with sedimentation and enhance its inhibitory effect on MPB production.

The increases in turbidity associated with sediment addition may have contributed to the reduction in MPB biomass. Turbidity levels remained elevated in the water column for three days after each sediment addition. This is similar to the persistence time of urban runoff plumes observed in coastal environments (Washburn et al. 2003, pers. obs.). Although the increase in turbidity would not have reduced light penetration to the sediment surface during low tide, it would have reduced it during high tide intervals, thereby reducing the photosynthetic rate of submerged MPB (Admiraal & Peletier 1980, MacIntyre et al. 1996, Wood & Armitage 1997).

Alternatively, the erosive forces of the suspended sediments may have increased the resuspension of diatoms near the sediment surface during high tides. A study examining the movement of resuspended diatoms from intertidal estuarine flats indicated that most of the resuspended diatoms are exported into deeper channels during the ebb tide (Lucas 2003). Therefore, if the sediment additions did lead to an increase the resuspension of MPB, this is likely to have resulted in a greater export of

MPB from the mesocosms during ebb tide flows. In this study, Chl *a* was not measured in the tidal water, but inclusion of this measurement in future experiments would provide useful insight into the validity of this model.

In previous chapters, it was proposed that the deposition of fine urban sediments may reduce the penetration of sunlight to partially buried diatoms during diurnal low tide, leading to a reduction MPB biomass. Benthic diatoms carrying out photosynthesis on the sediment surface during diurnal low tide may undergo a micro-migration into deeper sediments as a strategy to avoid photo-inhibition. This enables the diatoms to continue photosynthesis at lower light intensities (Underwood 2002, Van Leeuwe et al. 2008). Sunlight, however, has little penetration in fine, muddy sediments, therefore, the deposition of urban sediments may reduce the primary productivity of these partially buried diatoms (e.g., Underwood 2002). The light intensity of the mesocosms, however, was similar to that underneath the mangrove canopy. Therefore the diatoms in the mesocosms probably had no need to migrate into deeper sediments to avoid photo-inhibition.

### **5.5.2 Crab condition**

Crab HI showed no response to the addition of N or sediment. This result runs counter to the model that N addition would increase crab condition and sediment addition would decrease crab condition, offsetting the effect of N. The lack of response to N addition is likely to reflect the lack of response also displayed in MPB biomass. MPB is considered an important source of food for ocypodid crabs (France 1998, Bouillon et al. 2002a, Doi et al. 2005). The predicted increase in crab condition was based on the assumption that the addition of N would lead to an increase in MPB biomass, thus increasing the amount of food available to the crabs (Kennish 1997). However, the addition of N had no effect on MPB biomass and, therefore, no effect on food availability, thus explaining the lack of increase in crab condition.

Crab condition also, however, did not reflect the decline in MPB biomass, which did occur in response to sediment addition. This may have been because the crabs were able to utilise an alternative food source to living MPB. The positive trend displayed by phaeopigments in response to sediment addition indicates a higher proportion of dead MPB in the mesocosms that received sediment additions. The crabs may have obtained their nutrition from both the living and dead algae. In other words, rather than reducing the total food (MPB) pool, the sediment additions caused a shift in



the proportion of living to dead MPB consumed by the crabs, resulting in no change in crab condition. Alternatively crab condition may take more than one month to reflect changes in food availability, although Kennish (1997) reported monthly changes in the HI of the herbivorous shore crab, *Grapsus albolineatus*, in response to altered food sources.

This experiment did not demonstrate the cascading adverse impact of reduced MPB on crabs under additional sediments expected from survey results in Chapters 3 and 4. Through time, however, sedimentation is likely to reduce the total amount of MPB available to consumers, as less dead algae are produced from a diminishing stock of living MPB. Over a longer time scale, this overall decline in MPB is likely to be reflected in the condition of consumers, such as crabs. The corresponding declines in MPB biomass and crab condition reported in previous chapters are likely to reflect the long-term effects of sedimentation and, therefore, provide a better insight into the trophic cascade of its effects.

### **5.5.3 Conclusion**

The results from this study indicate that in turbid mangrove environments, nutrient enrichment at simulated urban runoff concentrations does not stimulate MPB production. Sediment additions appear to have a greater impact, causing reductions in MPB biomass, possibly due to nutrient repletion and MPB production being limited by light, in turbid mangrove environments. Throughout the time frame of this experiment, crabs appeared to be able to adapt to the reduction in MPB production, possibly by consuming an increased proportion of dead MPB, which may have occurred in response to sediment addition. The long-term effects of sedimentation will likely lead to reductions in the total amount of MPB available to consumers, as less dead algae is produced from a diminishing stock of living MPB. Therefore, crab condition is eventually likely to follow the decline in MPB biomass and demonstrate a similar cascade of response to that demonstrated in the previous two chapters.

## **Chapter 6. General discussion and conclusions**

### **6.1 Coastal urbanisation: scale and implications**

Estuarine environments and the ecosystem services they provide are threatened by increasing urbanisation of coastal areas. The doubling in human population over the last 50 years has led to the significant transformation of more than one third of the Earth's land surface (Vitousek et al. 1997). More than 60% of the urbanisation supporting this growing population is located within 100 km of the coast, imposing unprecedented stress on coastal ecosystems (Vitousek et al. 1997). People are lured to the coast by its natural beauty, relaxed lifestyle and recreational opportunities (Stimson & Minnery 1998). Yet the growing human population increases stress on estuaries and coasts by altering nutrient and pollutant loads, changing catchment hydrology and increasing sedimentation (Lee et al. 2006).

Perhaps the most radical landscape change associated with urbanisation is the replacement of permeable vegetated areas with impervious areas. Impervious surfaces such as roads, footpaths, and car parks accumulate urban pollutants and during rainfall convey these pollutants from the catchment via surface water channels, such as streams and rivers, into coastal waters and estuaries, impacting the water quality and ecology of these environments (Arnold & Gibbons 1996, Mallin et al. 2000, Holland et al. 2004, Gobel et al. 2007).

Increasing evidence shows that pollutants from urbanised catchments degrade the structure and function of receiving estuaries, impacting water quality, altering nutrient cycles and habitat complexity, reducing fishery production and ecosystem organisation, making the systems less resilient to further disturbance (e.g., Boesch 2000, Glasgow & Burkholder 2000, Inglis & Kross 2000, Holland et al. 2004, Lee et al. 2006). Yet few studies have assessed the relationship between these responses and one of the key drivers of urban impacts in receiving estuaries – impervious surfaces. A thorough understanding of this relationship is pivotal in the identification of ecological threshold limits which will assist urban planners in devising strategies which conserve the health of estuaries and the vital services they provide.

This thesis assessed the effects of urbanisation, measured as impervious cover (IC) on mangrove dominated estuaries around southeast Queensland by measuring responses in biota that may offer an early warning for ecosystem degradation.

In this chapter, I review the conclusions of the thesis and discuss their relevance to, and implications for, the monitoring and management of urban impacts in estuarine environments.

## **6.2 Summary of findings**

This thesis assessed whether urbanisation of coastal areas in southeast Queensland affected important biotic characteristics of the mangrove intertidal ecosystems in the region. I measured three indicators: (1) the  $\delta^{15}\text{N}$  of mangrove biota, (2) the biomass of MPB (microphytobenthos), and (3) the condition of mangrove crabs. The  $\delta^{15}\text{N}$  of mangrove biota was used as a tracer for urban sources of nitrogen (N), thus providing an indication of the permeation of anthropogenic nutrients in these ecosystems. The biomass of MPB provides information on the biotic responses to anthropogenic inputs and the potential for eutrophication, and the condition of crabs provides a static indication of the health of ecologically important consumers. I related these metrics to the extent of urbanisation, measured as IC, across a gradient of estuarine sites. These findings contribute to our understanding of how estuarine biota respond to current urbanisation practices, and in doing so, offer some insight as to how future urban design may be improved to mitigate urban impacts in estuaries.

### **6.2.1 Localised responses of mangrove biota indicate sources of urban influx within Lake Coombabah**

Elevations in crab  $\delta^{15}\text{N}$ , which were correlated with rainfall, at distances near and far from two of the influx sites indicated that urban sources of N are conveyed into the lake in urban runoff (Dillon & Chanton 2008). A more rapid recovery of  $\delta^{15}\text{N}$  to pre-rainfall values in crabs located farther away from the sites indicated a longer residence time of these nutrients in habitats closer to the influx sites. Enriched  $\delta^{15}\text{N}$  values in the tuxedo crab (*Australoplax tridentata*) and grey mangrove (*Avicennia marina*) around the entrance to East Creek (EC), which borders a STP, indicated that the STP contributes sewage derived N to the lake (Costanzo et al. 2001, Savage & Elmgren 2004, Schlacher et al. 2005). Whilst there is no deliberate discharge of treated sewage into the lake, possible groundwater seepage or overflow from the STP into EC, which flows into the lake, may connect the two. Greater temporal fluctuation in the condition of crabs near the EC influx site indicates that exposure to sewage N and other urban pollutants is having a localised impact on the condition of consumers in the lake (Underwood 1998).

Chapter 2 provided a useful indication of the sources of urban nutrients and the scale of impacts on biota within an urbanised estuarine lake. Because this work was completed within the one lake, it did not provide an assessment of the estuarine responses to urbanisation – in a general sense. Ideally the comparison of several urbanised and several pristine estuaries would enable this, however, pristine sites are scarce in southeast Queensland. Alternatively, an assessment of any relationship between biotic responses and urbanisation in estuaries surrounded by different levels of urbanisation would improve our broader understanding of how estuarine ecosystems respond to urbanisation.

### **6.2.2 Responses of mangrove biota to increasing urbanisation**

Chapters 3 and 4 assessed the relationship between increasing urbanisation and the responses of estuarine biota. The extent of urbanisation within estuary catchments was estimated using a census of the percentage of IC within each catchment (IC) (Arnold & Gibbons 1996, Holland et al. 2004). Many of the estuaries in southeast Queensland comprise lakes and creeks. So to investigate the generality of this relationship, the response of mangrove biota to increasing urbanisation was assessed using multiple lakes (Chapter 3) and creeks (Chapter 4) around the region.

These chapters indicated that biota in estuarine lakes and creeks with greater catchment urbanisation were in poorer condition. Declines in MPB biomass and crab condition indicated that stressors, which inhibit MPB production and consumer condition, such as sediments or metals, were the predominant pollutants in urban runoff affecting the biota of estuaries in the region (MacIntyre et al. 1996, Moreno-Garrido et al. 2003, Elumalai et al. 2005). These indicators were combined to generate a composite index of biotic condition (BCI) that was negatively related with urbanisation, both in lakes and creeks.

In the estuarine lakes, this relationship was only evident when IC had been adjusted for the size of each catchment relative to the size of each lake. The ratio between these two variables is likely to influence the sensitivity of estuaries to catchment activities; large estuaries with small catchments are generally more resilient to impacts associated with catchment disturbance and vice versa (Horton & Eichbaum 1991). This relationship is particularly likely to apply to estuarine lakes, which have slow hydrological flow rates and long water residence times, making the size of the water body an important factor in the dilution of pollutants (William 1986, Soballe &

Kimmel 1987). The adjusted IC index (AIC) explained 79% of the variation in the BCI measured in the lakes.

In the estuarine creeks, the biotic condition index showed a more linear decline with increased urbanisation (indicated by IC). Without having to be adjusted for differences in the catchment to estuary size ratio, IC explained 61% of the variation in biotic condition. When IC was adjusted for differences in the catchment to estuary size ratio the biotic health of the creeks showed no obvious trend with urbanisation. This suggests that for creeks, which tend to have faster flow rates than lakes, the size of the water body has less influence on the dilution of pollutants (Soballe & Kimmel 1987).

Enrichment patterns of  $\delta^{15}\text{N}$  values in mangroves and crabs indicated that septic tanks and STPs within the estuaries' catchments made a greater contribution to urban N in the creeks and lakes than urban runoff (Valiela et al. 2000, Savage & Elmgren 2004, Dillon & Chanton 2008). However, small elevations in the  $\delta^{15}\text{N}$  of biota in some of the estuarine creeks, which were not exposed to sewage effluent, indicated some contribution of nutrients from urban runoff. The assimilation of these urban sources of N did not appear to be having any effect on MPB biomass or crab condition in either the lakes or the creeks. Rather, the decline in these indicators was best explained by the extent of catchment urbanisation (AIC and IC), indicating that pollutants in urban runoff, such as sediments or metals, may have a greater impact on the biotic condition of these systems than nutrients from urban sewage.

As strategies to mitigate urban stormwater inputs to estuaries are implemented, the removal of pollutants such as sediments could lead to sewage N inputs having a greater effect on biota. This could make eutrophication problematic in estuaries which receive N from other urban sources, such as STPs. An understanding of the interactive effects of urban N and stormwater inputs in estuaries is vital for the effective management of coastal urbanisation and the preservation of estuaries. In Chapter 5 I investigated the interactive effects between N and sediment additions on MPB biomass and crab condition.

In Chapter 3, the temporal variability of the indicators measured at the whole-lake scale showed no relationship with urbanisation. This is likely to have been due to a combination of below average rainfall throughout the study and the size of the lakes diluting any temporal changes associated with urban runoff events. Detecting changes in temporal variance at these larger spatial scales is likely to require substantial urban

runoff events. Therefore, a sampling period spanning a number of years may be necessary to capture such stochastic events.

### **6.2.3 Biotic responses to nitrogen and sediment additions**

Urban runoff often delivers nutrients such as N and phosphorous essential for MPB production (CWP 2003, Lever & Valiela 2005). However, urban runoff is also associated with increased sediment loads, which make water turbid, blocking sunlight, and can also potentially smother MPB. Therefore, while both are characteristic of urban runoff, nutrients and sediment potentially act antagonistically on estuarine MPB. In Chapter 5, I used a controlled laboratory mesocosm experiment to test antagonistic effects of sediment and N additions on mangrove biota (MPB biomass and crab condition) over a one-month period.

MPB biomass showed no response to N enrichment, indicating that the mud in the mesocosms was already nutrient replete. Therefore MPB growth may have been limited by other factors such as light, phosphorus, or silica levels (Conley 2000, Stutes et al. 2006). Previous studies in muddy estuarine environments have also documented no response in MPB production to nutrient addition, indicating that MPB growth in these turbid environments is limited by light rather than N (e.g., Barranguet et al. 1998, Underwood et al. 1998, Stutes et al. 2006). Any influence that urbanisation has on this phenomenon due to the increased transfer of sediments via urban runoff into these environments remains untested. In any case, this light limitation is likely to make these environments more susceptible to the effects of urban pollutants that reduce light availability and inhibit MPB production. The lack of increase in crab condition to N additions may have been associated with the lack of increase in food availability (MPB biomass).

The reductions in MPB biomass, which occurred in response to the sediment additions, may have been caused by reductions in light penetration to submerged algae during the simulated high tides, resulting in reduced photosynthetic rates. Alternatively, the increased suspended sediments may have increased the erosion and resuspension of MPB in surface sediments during the simulated high tides, resulting in a greater export of MPB from the mesocosms in tidal water. This could be tested in future experiments by measuring MPB biomass in the water column as well as on the sediment surface.

During the time frame of the experiment, the decline in MPB did not influence crab condition. The crabs may have consumed an increased proportion of dead MPB,

which appeared to occur in response to the sediment additions. In the longer term, however, sedimentation is likely to reduce the total amount of MPB as less dead algae is produced from a declining stock of living MPB, in addition to the potential export of algae from the benthos into the water column. This reduction may then cascade through the food web and lead to declines in crab condition, similar to those observed in Chapters 3 and 4.

At the levels applied to the mesocosms, which were similar to those reported in urban runoff, MPB biomass was affected more by sediment than N additions (Drapper et al. 2000, Barry et al. 2004). These results, together with those reported in the literature (e.g., Stutes et al. 2006), suggest that increased sediment loads have a greater impact on biota than nutrient enrichment in turbid estuaries, where MPB production may be limited by light, particularly during tidal inundation.

### **6.3 Mangrove biota provide an early warning of ecosystem degradation in urbanised estuaries**

#### **6.3.1 Urban sewage affects estuarine nutrient sources**

The assimilation of isotopically enriched urban N indicates that biota in more urbanised estuaries experience greater exposure to anthropogenic nutrient sources. Anthropogenic sources of N associated with urbanisation include: treated sewage, groundwater N from septic systems and urban runoff (Savage et al. 2002, Valiela & Bowen 2002, Dillon & Chanton 2008). The  $\delta^{15}\text{N}$  values of mangrove biota demonstrated clear patterns of enrichment linked with the presence of septic tanks, STPs, and the direct discharge of sewage. This indicates that urban sewage is altering the nutrient sources and potentially, the nutrient cycles of estuaries, regardless of the extent of surrounding urbanisation.

While  $\delta^{15}\text{N}$  provides a convenient fingerprint for urban N sources, it does not necessarily signal an increase in the total N loads entering estuaries. Rather, it may signal a switch to the use of urban N sources as natural sources of N have been removed due to the replacement of vegetated areas with impervious areas. This switch *per se* may have an effect on the health of estuarine biota, although MPB biomass and crab condition showed no obvious response to the assimilation of urban N in Chapters 3 and 4. Instead, the decline in MPB biomass with increased urbanisation shown in these chapters indicates that pollutants delivered in urban runoff, which inhibit primary productivity, outweigh any effects of sewage N in estuaries. The results from Chapter

5, together with previous studies (e.g., Stutes et al. 2006) indicate that in turbid mangrove environments, MPB production is limited more by light than nutrients.

As the human population of southeast Queensland continues to grow, however, greater amounts of nutrients from treated sewage are expected to enter estuaries in the region (EPA 2007). Whilst a greater amount of this effluent is likely to be reused for potable purposes in order to meet the growing water demands of the region, the additional treatment of this is likely to lead to the release of increased concentrations of sewage pollutants into estuaries (QWC 2008). Thus, the enrichment of  $\delta^{15}\text{N}$  observed in biota in this thesis may provide an early warning for eutrophication in the future, particularly if future stormwater mitigation practices lead to the removal of sediments that counteract the effects of nutrients. This could potentially result in the estuaries becoming N limited, rather than light limited.

In addition, other pollutants in sewage and recycled water effluent, such as heavy metals or synthetic oestrogens, are likely to impact estuarine biota in ways that were not measured in this study. These pollutants can lead to endocrine disruption and tissue damage in biota (Depledge & Billingham 1999, Schlacher et al. 2007). Therefore, indicators that incorporate these measures should also be considered before drawing conclusions about the effects of urban sewage on estuarine biota.

#### **6.4 Implications for the health of urbanised estuaries**

The declines in biotic condition signify that urbanisation is starting to impact the health of estuarine ecosystems around southeast Queensland. MPB contribute importantly to the structure and function of estuarine ecosystems. The extracellular polymeric substances (EPS) produced by MPB help to stabilise surface sediments (Wolfstein & Stal 2002). Therefore declines in MPB biomass may lead to increases in the resuspension of surface sediments, providing a feedback loop that increases turbidity, the resuspension of remaining MPB, and net reduction in the biomass of MPB. MPB are also a vital food source to many estuarine consumers. Therefore, reductions in MPB biomass are likely to reduce the system's ability to provide a carbon base to the estuarine food web. The declines observed in crab condition may already reflect this. The decline in crab condition indicates a reduction in the crabs' sustenance stored in the hepatopancreas for repair and maintenance, thus reducing the resilience of crabs to additional disturbance.

Brachyuran crabs fill important roles in estuaries as ecosystem engineers (Jones et al. 1994, Kristensen 2008). Their feeding and burrowing activities enhance important



biogeochemical processes and influence mangrove production and forest structure (Smith 1987, Fenchel 1996, Kristensen & Alongi 2006, Cannicci et al. 2008, Lee 2008). Their consumption of leaf litter forms an intermediate link between mangrove detritus and other deposit feeders within estuaries (Lee 1997, Werry & Lee 2005, Nordhaus & Wolff 2007, Lee 2008). The crabs and their reproductive outputs are also thought to provide a significant trophic link between estuarine producers and predators (Hollingsworth & Connolly 2006, Lee 2008). Thus declines in their populations are likely to affect the structure and function of mangrove ecosystems. The reductions in crab condition are likely to increase the susceptibility of local populations to extinction in urbanised estuaries due to disturbances such as flooding and pollutant spills.

Continued coastal urbanisation is likely to reduce the capacity of these estuaries to provide important ecosystem services including the conversion of solar energy and nutrients into food, the provision of nursery habitats for important crabs and fish, wintering grounds for migratory shorebirds, and areas of aesthetically pleasing natural environments for human recreation (Valiela et al. 2001, Valiela & Cole 2002, Dahdouh-Guebas et al. 2005, Nagelkerken et al. 2008). Intelligent urban planning which strives to maintain the natural hydrology of catchments will assist in conserving these ecosystems.

## **6.5 How did $\delta^{15}\text{N}$ , MPB biomass, and crab condition measure up as biotic indicators?**

In this thesis the  $\delta^{15}\text{N}$  of mangrove biota, MPB biomass and crab condition detected changes in nutrient sources, reduced benthic primary production and reduced consumer condition. The  $\delta^{15}\text{N}$  of mangroves and crabs provided a useful tracer for the assimilation of nutrient sources derived from urban sewage (McClelland & Valiela 1998, Savage 2005, Schlacher et al. 2007). However, the  $\delta^{15}\text{N}$  values of mangrove biota did not provide any significant indication of estuarine responses to catchment imperviousness, or of the assimilation of N from urban runoff, contrary to the findings of Dillon & Chanton (2008).

The combination of MPB biomass and crab condition into the BCI, however, provided a convenient, multi-trophic indication of response to catchment imperviousness. The combination of ecologically relevant indicators into multi-metric indices has, in recent years, received attention as a practical means of linking ecosystem level responses with anthropogenic disturbances (Galloway et al. 2004). Fish community metrics based on measures of species richness, fish abundance, trophic

composition and nursery function have been reported to reflect degradation of coastal habitats linked with coastal development (Bilkovic & Roggero 2008). In Chesapeake Bay a Bay Health Index, which combined measures of water quality, including Chl *a*, dissolved oxygen and secchi depth with biological measures, representing the abundance and diversity of phytoplankton, benthic invertebrates and submerged aquatic vegetation, showed a negative relationship with increased development and agricultural landuse in surrounding watersheds (Williams et al. 2009). Recent research evaluating the performance of biodiversity based multimetrics with that of individual indices showed that multimetric indices had a greater success rate in identifying habitat disturbance (Ranasinghe et al. 2009).

The BCI used in this study offers a cost effective means of identifying biotic impacts linked with early signs of ecosystem degradation in estuaries around southeast Queensland (Depledge & Fossi 1994, Rapport 1998b). From a management perspective it would be useful to derive a threshold value for the BCI, which in turn could be used to identify a threshold for IC, based upon the relationships observed in this thesis. Providing these thresholds are set at values which occur before notable declines in ecosystem structure and functionality occur, they could provide a powerful monitoring tool – enabling the detection and mitigation of impacts before irreversible ecosystem degradation takes place. However, making an objective choice of a meaningful threshold based upon the relationship between IC and BCI is challenging, without first understanding the implications of this relationship at the broader ecosystem level. The next logical step is to gain some understanding of this by measuring relationships between the BCI and measures of ecosystem health, such as species diversity, ecosystem functionality and the provision of ecosystem services. Once these relationships are understood, threshold values can be selected for BCI and IC, which enable preservation of the current value of estuaries and the ecosystem services they provide (Depledge 1994).

Whilst the responses of crab HI strongly reflected the declines in MPB biomass with greater catchment urbanisation, it should be noted that crab HI did not show as clear a response to urbanisation as MPB. This indicator showed a greater degree of variability within the field sites, probably due to its sensitivity to natural factors such as the metabolic costs of reproduction, foraging (costs and benefits), and competition (e.g. Kyomo 1988). Future monitoring studies applying this indicator should increase experimental power by sampling a greater number of crabs. However, in situations

where power is limited by the number of field sites (as was the case in Chapters 3 and 4), the use of crab HI should be limited to studies in which the effect size of the impact is likely to be large ( $> 0.4$ ).

## **6.6 Recommendations for the future monitoring of estuaries**

In cases where impacts are initially subtle, the use of sensitive biomarkers such as cellular energy budgets and histopathology measures in estuarine biota may provide an early signal of biotic response (Verslycke et al. 2003, Schlacher et al. 2007). These biomarkers may be useful for the early detection of urban runoff and treated sewage in urbanised estuaries. Sewage effluent contains a mixture of nutrients, metals and endocrine disrupting chemicals which can alter the energy budget of animals and cause tissue damage (Depledge & Billingham 1999). Schlacher et al. (2007) proposed the use of histopathology in estuarine fish as a powerful biomarker for understanding the effects of urban sewage on estuarine ecosystems. The cellular energy budget of shrimp has also shown clear responses to endocrine disruptors such as tributyltin (Verslycke et al. 2003). The use of such direct, tangible measures of biotic health is appealing as they improve the ease of communication of ecological impacts between ecologists and managers. However, before such measures can be applied to monitor impacts at the ecosystem level, we must obtain a thorough understanding of their relationship with ecosystem health.

Whilst biotic indicators may offer early detection of impacts before ecosystems switch to degraded states, they do not provide a direct estimate of the state or health of the ecosystem. Many previous efforts to detect impacts to ecosystem health have relied on structural measures such as species diversity or signs of ecosystem distress, such as eutrophication. These measures offer an ecosystem level approach, but they detect impacts only when considerable ecosystem degradation has already occurred. There is a need for indicators that can detect impacts at the ecosystem level before such degradation has occurred. Measurement of the rates of ecological processes, or 'eco-assays', has been proposed as a novel approach of detecting subtle changes in the performance of an ecosystem (Fairweather 1999). These may capture changes in the level of activity or 'vigour' in ecosystems exposed to urbanisation and may include the measurement of significant ecological processes, such as competition rates, predation, decomposition, or scavenging (Webley 2008). The assessment of carbon pathways and energy transfer efficiency between specific trophic levels may also be useful in

detecting impacts as the suite of species that drive these functions changes in response to disturbance (Valiela 1995, Walker 1995, Rapport 1998b).

Whilst closely interdependent with ecosystem vigour and organisation, in terms of ecosystem management, resilience has recently become a favoured integrated measure in understanding the thresholds for human impacts in ecosystems (Nystrom et al. 2000, Hughes et al. 2005, Thrush et al. 2008). When an ecosystem's resilience is exceeded, it may switch to an 'alternate', or degraded state (Rapport et al. 1998, Scheffer et al. 2001). An example of this is the overfishing of herbivorous fish and nutrient enrichment in the Caribbean coral reefs. This led to a switch from a coral community to an algae-dominated community. Subsequently, a sea urchin which had previously competed with the herbivorous fish increased in numbers and was able to fill the role of the fish and control the algae (Nystrom et al. 2000). However, when a pathogen reduced the sea urchin population the system became algae-dominated again as there were no additional grazers to control the algae. This altered state is considered difficult to reverse because adult algae are less palatable to herbivores and the algae prevent settlement of coral larvae (Nystrom et al. 2000, Scheffer et al. 2001). Thus, as the system organisation (diversity and redundancy of grazers) and vigour (rates of competition between grazers) reduced, the ecosystem's resilience to an additional, stochastic disturbance (sea urchin pathogen) was reduced, resulting in the system changing to an altered state.

Transitions into degraded or altered states are thought to be preceded by periods of declining ecosystem resilience. Therefore, detection of reduced resilience is likely to provide an early warning of ecosystem degradation at the ecosystem level, while the chances of restoring the ecosystem are still good (Scheffer & Carpenter 2003, Carpenter & Brock 2006). Recently, the variability of ecosystems has been proposed as an indicator of resilience (Scheffer & Carpenter 2003, Hughes et al. 2005, Oborny et al. 2005, Brock & Carpenter 2006, Litzow et al. 2008). Ecologists have suggested for the last 40 years that disturbances increase the variability of ecosystems (Odum et al. 1979, Underwood 1998). Increases in the temporal variability of algal biomass have been documented in response to nutrient enrichment (Cottingham et al. 2000). More recently Litzow et al. (2008) documented that the spatial variability in the ratio of cod to prey abundance increased significantly in the three years leading up to the collapse of the North Atlantic cod fishery.

In this thesis the temporal variability of crab condition showed a significant localised increase at an urban influx site adjacent to a STP but no clear response to increasing urbanisation at the whole-lake scale. This was potentially due to the size of the lakes diluting any localised temporal changes in crab condition around influx sites and the scarcity of substantial storm runoff events during this study. However, measurement of temporal and spatial variability of key ecosystem parameters in estuaries using longer-term data sets may provide a more effective means of detecting changes to resilience in urbanised estuaries. In addition to this, measurement of recovery rates may also provide an indication of resilience in estuaries (Thrush et al. 2008). Furthermore, obtaining a thorough knowledge of the diversity of important functional groups, as well as the extent of species redundancy within these groups, will enable assessment of the potential resilience of estuaries (Walker 1995, Ehrlich & Walker 1998).

## **6.7 Impervious cover as an indicator of urban impact**

Impervious cover is a landscape feature that provides an indication of the cumulative impacts of catchment urbanisation on receiving water bodies (Arnold & Gibbons 1996, Lerberg et al. 2000, Mallin et al. 2000, Holland et al. 2004). The replacement of natural, pervious areas with impervious areas affects the processes that connect terrestrial catchment materials with aquatic systems. IC increases the rate at which stormwater is delivered to waterways, side-stepping natural biofiltration processes, therefore increasing the amounts and changing the temporal patterns of pollutant loads delivered into waterways (Arnold & Gibbons 1996, Holland et al. 2004).

Most previous efforts to understand the ecological impacts associated with IC have been done in freshwater systems and have indicated impacts when IC exceeds 10 – 20% (Arnold & Gibbons 1996, CWP 2003). The few studies that have focused on these responses in estuarine systems indicated similar relationships between IC and biotic indicators with impacts occurring when IC exceeded 20 – 30% (Lerberg et al. 2000, Holland et al. 2004). These studies, however, were done in macro-tidal creeks, dominated by marsh grass, in South Carolina, USA. Therefore, until this study, it was unknown if this relationship applied to other estuarine systems with smaller tidal ranges and less flushing.

The findings of this thesis support the model that estuarine biota become progressively more impacted as IC increases. Certain system attributes, however, appear to influence this relationship. In estuarine lakes (Chapter 3), the relationship between IC and biotic condition was only evident when IC was adjusted for the catchment to estuary size ratio of each lake. The biotic response to increased IC was then best explained as a power relationship, with rapid declines in biotic condition occurring at 4.2% IC. This decline in biotic condition at such a low level of IC reflects how differences in the sensitivity of specific systems may influence the relationship. The decline in condition occurred in the lake with the second largest catchment to lake area ratio, indicating that smaller estuarine lakes with larger catchments are more sensitive to catchment urbanisation (Horton & Eichbaum 1991). In Chapter 4, the response in biota to increased IC was more linear, this may have been due to hydrological differences between the creeks and the lakes. The faster flow rates of the creeks may contribute to making the biotic response to urbanisation more gradual as urban pollutants would not have as long to accumulate within the creeks, compared to in the slower flowing lakes (Soballe & Kimmel 1987).

While IC is a useful tool for estimating urban impacts in receiving estuaries, a wide range of methods are used to quantify it. These include: point sampling from aerial photographs, various GIS techniques, remote sensing of land cover, and applying literature values of IC to specific land use categories (Arnold & Gibbons 1996, Wu & Murray 2003, Holland et al. 2004, Walsh et al. 2004). The comparability of these various estimates of IC is likely to vary (Holland et al. 2004). Future research examining estuarine responses to increasing urbanisation would benefit from using a standardised database of IC, or a standard method of quantifying IC.

## **6.8 Solutions**

Urban growth in coastal areas of southeast Queensland is predicted to continue for at least the next five decades (LGP 2006). In order to achieve sustainable urban growth that conserves important coastal ecosystems and their services, Beach (2002) and Holland et al. (2004) suggest actions at three levels: regional, neighbourhood and local. Regional-scale actions need to determine where new development can and cannot occur. This should take into consideration differences in the sensitivity of specific estuarine systems, as well as the extent of IC and the extent of drainage connectivity between this and the receiving waterways (Walsh et al. 2005). At the neighbourhood

scale, new developments need to implement designs that reduce stormwater runoff volumes and pollutant loads, and minimise the construction of new impervious surfaces. At the local scale, public education efforts need to occur to increase awareness of the connection between catchment activities and the health of coastal environments.

In recent years actions have been implemented at the neighborhood and local scales around Australia. These efforts include the adoption of a water-sensitive urban design approach (WSUD) in new housing developments, where emphasis is placed on reducing stormwater runoff and re-using stormwater for non-drinking purposes such as irrigation. In these developments, drainage systems are designed to catch stormwater from roofs and adjoining impervious areas. This is then directed to various onsite biofiltration and UV treatments, before being reused for irrigation and other purposes (DEH 2002). In car parks and commercial zones, porous paving is being trialed to reduce stormwater runoff and provide filtration of pollutants so that stormwater can be used to irrigate adjacent parks. Bio-retention basins and artificial wetlands have also been constructed in “water sensitive” urban areas to remove pollutants from stormwater (DEH 2002). So far, however, little research has been done to assess any of the beneficial effects of WSUD in receiving waterways (Fletcher et al. 2008).

At the local scale, effort is also being made by local governments and integrated catchment management groups, such as Southeast Queensland Healthy Waterways, to improve public education about sustainable stormwater management. Conservation of existing riparian vegetation and forested areas within catchments can help to reduce stormwater runoff. On average, forest canopies around southeast Queensland have been reported to intercept 47% of rainfall (Hutley et al. 1997).

While measures to mitigate urban runoff are starting to be implemented, they need to be maintained at a rate that keeps up with the rate of urban growth. There is also a need for a holistic approach to managing urban stormwater and other urban pollutants, including sewage effluent. N loading from the release of sewage effluent is predicted to increase by 50% within the next 20 years (EPA 2007). The mitigation of urban stormwater may reduce the turbid condition of urbanised mangrove-dominated estuaries, potentially making them more susceptible to the effects of increasing N loads from sewage sources. Ongoing monitoring of the regions estuaries using sensitive ecosystem level and biotic indicators is vital in ensuring that urban management practices effectively conserve the health of these ecosystems and the services they provide.

## 6.9 Summary and recommendations

Using a suite of biotic indicators, this thesis detected early signs of degradation in estuaries faced with growing urbanisation pressures. The  $\delta^{15}\text{N}$  measured in mangrove biota indicated a change in nutrient sources that was associated with the presence of sewage infrastructure. At present, this switch in nutrient sources does not appear to affect the condition of estuarine biota. Potentially, because other factors such as light have a greater influence on primary production. As the southeast Queensland population continues to grow, however, the biotic responses to the increasing release of sewage nutrients and pollutants from recycled water treatment should be monitored so that ecosystem changes can be detected early.

Declines in the biomass of MPB indicated that pollutants in urban runoff are decreasing the benthic primary productivity of estuarine environments. MPB are an important energy source in the estuarine food web, their removal is likely to reduce the capacity of these ecosystems to support both ecologically and commercially valuable estuarine consumers. Similar declines in crab condition indicated that urban pollutants are having a negative impact on estuarine consumers either directly or due to a trophic cascade of impacts associated with the decline in MPB biomass. Reductions in condition are likely to reduce the resilience of individual crabs to disturbance, potentially making their populations more vulnerable to local extinction in urbanised estuaries. The removal of these important ecosystem engineers is likely to alter the structure and function of mangrove ecosystems, reducing their resilience to further disturbances.

Urban growth in southeast Queensland is set to continue over the next 50 years. While sustainable urban design practices are being implemented to reduce the flow of urban runoff into estuaries, ongoing monitoring of the health of estuaries is pivotal in ensuring the sustainable management of coastal urban growth. The biotic indicators measured in this thesis offer a convenient means of monitoring estuarine responses to future urbanisation practices. The application of these indicators with other novel biomarkers and ecosystem health measures, such as eco-assays and the variability of key ecosystem components, will assist in providing a more holistic assessment of the state and performance of estuarine ecosystems.





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