Mackay Mangrove Dieback

Investigations in 2002 with Recommendations for Further Research, Monitoring and Management

NC Duke
AM Bell
DK Pedersen
CM Roelfsema
LM Godson
KN Zahmel
J Mackenzie
S Bengtson-Nash

The University Of Queensland
AUSTRALIA

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**Norman C. Duke, Alicia M. Bell, Dan K. Pedersen,
Chris M. Roelfsema, Lloyd M. Godson,
Katherine N. Zahmel, Jock Mackenzie
and Susan Bengtson-Nash**

**Marine Botany Group
Centre for Marine Studies
The University of Queensland**

THE UNIVERSITY
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**Report to the
Queensland Department of Primary Industries, Northern Fisheries Centre
and
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Contact Details: Dr Norman C Duke
Marine Botany Group, Centre for Marine Studies
The University of Queensland, Brisbane QLD 4072
Telephone: (07) 3365 2729
Fax: (07) 3365 7321
Email: n.duke@uq.edu.au

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COVER PAGE FIGURE: Aerial view of severe *A. marina* dieback along Barnes Creek, Pioneer River Estuary, Mackay region. Artwork: Diana Kleine, Marine Botany Group.

Executive Summary

Serious Dieback of Mangroves in Mackay Region

Dieback of mangroves in the Mackay region of Queensland Australia continues to be serious and progressive. This distinctive and rare kind of dieback is severe and species-specific, affecting ~50 km^2 of mangroves in five adjacent estuarine systems spread along 30 km of coastline, centred around the Pioneer River estuary. After 2 years, mangrove plants, notably *Avicennia marina* (the common mangrove tree), continue to show signs of unusual poor health and stress, and dead trees are not being replaced by new seedling recruits. Irrespective of the cause, all evidence indicates these important coastal estuarine habitats are in serious decline. As the situation worsens, the implications for adjacent marine ecosystems like seagrass beds and coral reefs are immense. It is imperative to discover the cause.

This report summarises the current status of research into this very serious environmental problem addressing a broad range of issues concerning the impact (defining the extent and condition of mangroves in the region), the cause (identifying correlates and effects, and isolating the most likely causative agents), the implications (recommending regular monitoring of the situation including key secondary consequences), and the need for urgent, but appropriate, management action. We report detailed new research findings gathered during 2002 in three investigative components, including: field and aerial surveys in the Mackay region; preliminary ecotoxicology trials in a planthouse; and comparative field investigations in 2 North Queensland river systems. These findings provide significant new evidence, which further implicate herbicides, particularly diuron, as the chief factor causing this instance of severe dieback of mangroves in Mackay region.

The consequences of dieback in the Pioneer River estuary were also more noticeable and serious in 2002. For example, sediment erosion appears to have accelerated in higher mudflat areas denuded of mangroves, and this sediment had deposited in lower mangrove areas along creek edges. In this way, burial of essential breathing roots was a secondary consequence of dieback, which meant other mangrove species might also be threatened. Furthermore, plant pathogen effects were also considered likely secondary consequences since they attack plants stressed by other agents.

Dieback Affects Most Mangrove Areas in Mackay Region

Mangrove dieback in estuaries of the Pioneer River, Bakers Creek and McCreadys Creek were mapped from aerial photographs taken in September 2002. These were used to quantify the extent and degree of damage at that time, and to establish a baseline from which to identify change in the future. Based on careful interpretation of aerial images, there were several notable observations. Dieback predominantly affected *A. marina*. In the Pioneer estuary, *A. marina* grew in mixed associations with other mangrove species in about 57% of the total mangrove area. Around 97% of *A. marina* areas were affected by severe and moderate dieback. Little or no dieback was observed in areas without *A. marina*. Affected *A. marina* trees were distributed from low water along creek margins to high water at terrestrial margins and from the river mouth to the upstream limits around Fursden Creek.

Since only *A. marina* had been affected, the condition of this mangrove species was taken as an indicator of the presence and effect of the dieback agent. This observation was affirmed in the field survey, which found most (97%), if not the entire mangrove area of the Pioneer River estuary was affected by the dieback agent in 2002. By comparison, both Bakers and McCreadys Creeks were less affected at 61% and 17% respectively of *A. marina* forests.

Mangrove Health Correlated with Herbicides in Sediments

Field studies in 2002 used a number of key indicators of mangrove health to assess a range of possible causative agents identified in the preliminary survey 2 years earlier. Based on these studies, the chief agents likely to cause the serious dieback, included: sediment burial, excess nutrients, excess heavy metals, and excess herbicides. During 2002 three estuaries were sampled including the Pioneer River, Bakers Creek and McCreadys Creek. This was done to address the key question of whether there might be a common agent correlated with dieback in each of these estuaries.

Breathing roots, or pneumatophores, of *A. marina* were taken as indicators of sediment burial and erosion. Root burial, however, was not correlated with severity of dieback in mangrove trees or mangrove health in either of the 3 estuaries. Similarly, there were no correlations between mangrove health and other toxic chemicals like heavy metals (notably Pb, Hg, Mn, Cu, Cd) and excess nutrients (N and P).

In contrast, there were significant correlations between concentrations of diuron in sediments and mangrove health shown in *A. marina* plants. Poor mangrove health and severity of dieback were shown as declining levels of chlorophyll in mature canopy leaves and decreasing proportions of healthy seedlings. These indicators showed the plots with greater levels of diuron in sediments had progressively poorer mangrove health and fewer healthy seedlings in respective estuaries.

In general, areas of severe mangrove dieback had herbicide levels up to 8.2 µg ai/kg of sediment, over twice the concentration found 2 years earlier in Barnes Creek in Pioneer River. Maximum levels in the 3 estuaries ranged between 6-8 µg diuron /kg sediment. Furthermore, either degradation rates of diuron in mangrove sediments were unexpectedly slow, or herbicide levels were replenished, because sites re-sampled after 2 years had similar levels in 2002. This was unexpected since reported degradation rates of diuron suggested break-down of the active ingredient occurred in less than one year.

The major source of herbicides in mangrove sediments was undoubtedly the adjacent agricultural lands of the surrounding catchment areas. Levels of herbicides in creek water from river mouths to upriver freshwater sources showed concentrations were highest in upriver areas, especially in agricultural drains flowing into the estuary upstream. Herbicide concentrations in free flowing drain waters were up to 1.1 µg/L in Pioneer River and Bakers Creek. In these drains, *A. marina* plants were either absent or if present they showed signs of poor health and condition, including yellowed leaves and deformed breathing roots. Diuron at these concentrations was reported previously to reduce seagrass growth and survival.

Mangroves Negatively Affected by Herbicides in Ecotoxicology Trials

In preliminary planthouse trials, seedlings of 4 mangrove species including two salt-excreters (*A. marina* and *Aegiceras corniculatum*) and two salt-excluders (*Rhizophora stylosa* and *Ceriops australis*) were treated with a range of concentrations of the herbicides diuron, ametryn and atrazine. Herbicides were applied to the roots of potted plants via water and sediments. Species were ranked by their overall sensitivity to herbicides with *A. marina> A. corniculatum> R. stylosa> C. australis*. These results concur with field observations from Mackay region where only *A. marina* was affected out of the ~20 species present.

Herbicides were ranked also by their toxicity to the 4 mangroves, from most toxic to least harmful, with diuron> ametryn> atrazine. High concentrations of herbicides were used in these initial trials to quickly answer two chief questions. First, to learn whether mangroves were affected by herbicides applied to their roots. Secondly, to establish whether *A. marina* was more sensitive than other species. After approximately 3 months, field-comparable concentrations of diuron were starting to affect *A. marina* health also. Longer-term studies are required urgently to fully investigate the effects of doses found in field sites. These trials support the idea that physiological differences

explain the characteristic species-specific dieback response observed in the Mackay region, and that different mangrove species have different susceptibilities to toxic pollutants.

Comparative River Studies of Mangrove Condition and Presence of Herbicide

Two river systems in north Queensland, Daintree and Johnstone, were surveyed in 2001 and 2002 to compare with the 3 estuaries in the Mackay region. Both rivers also had notable cane producing areas, although total crop areas varied considerably, being around 32 km^2 and 252 km^2 respectively. In comparison, the Pioneer River catchment reportedly had around 219 km^2 crop area.

In Daintree River estuary, *A. marina* was distributed upriver from the river mouth to ~50% of the mangrove range upstream. Diuron levels in mangrove sediments within this range of *A. marina* (sampled at 25-50% upriver position) were relatively low (0.1-1.1 µg/kg), and *A. marina* trees appeared healthy in this estuary.

In Johnstone River estuary, there were relatively few *A. marina* trees and these were healthy. However, in this case, *A. marina* extended only 25% upriver relative to other mangrove species. Diuron levels in the river were higher (0.4-5.2 µg/kg) especially in upstream sediments. In upriver area (sampled at 25-50% from the mouth), herbicide concentrations were curiously highest (> 2.5 µg/kg) where *A. marina* was absent, and lower (< 1.1 µg/kg) in downstream mangroves (0-25%) where *A. marina* was present. The question was, did the absence of *A. marina* further upriver in the Johnstone suggest this species had died many years earlier, or was the natural upriver distribution much less in this river for other reasons.

Unlike the two northern estuaries, *A. marina* dominated the Pioneer River estuary and appeared to extend upriver from the mouth to the mangrove limit. Furthermore, concentrations of the herbicide diuron in sediments beneath *A. marina* trees were 6-8 times higher in the Mackay region, compared with the relatively low levels, ~1 µg/kg or less, in the northern rivers.

Conclusions and Implications

There are several fundamental observations to be made from these findings in 2002:-

- There was serious and unprecedented severe dieback of mangroves in the Mackay region, notably affecting *A. marina* more than other species.
- There were unacceptable levels of herbicides in mangrove sediments in the region.
- Levels of the herbicide diuron had not diminished after 2 years, and higher levels were detected in 2002.
- The dieback had gotten worse and the extent has increased to affect most mangrove areas in the region.
- Correlative assessments (at >95% certainty) of mangrove condition and health in the field showed there was one likely agent, namely herbicides (particularly diuron).
- Planthouse trials demonstrated that mangrove plants were affected by herbicides and that *A. marina* was the most sensitive species of 4 tested.
- Diuron concentrations in the Mackay region were 6-8 times higher than in *A. marina* mangrove areas of the Johnstone River, an area of similarly intense cane cultivation and herbicide use.
- There were apparent secondary consequences of dieback including potentially massive sediment mobilisation and displacement taking place in estuarine areas, notably in the Pioneer River estuary.
- There were likely to be profound affects on associated flora like seagrass beds, and dependent fauna like prawns, fish and birds.
- There were likely to be profound effects on nearshore habitats, like coral reefs, due to both herbicides and declines in water quality with increased turbidity, nutrients and sediment deposition.

Urgent action is recommended: 1) to reduce the amount of herbicides depositing in mangrove sediments of the Mackay region, 2) to learn more about the progress, fate and implication of mangrove dieback, and 3) to monitor the condition of mangrove habitat annually until the situation improves with cessation of further dieback and successful recruitment and growth of seedlings. These three components need to be included in an adaptive management framework for coastal environment protection and sustainability for the region.

Contents

Key Findings and Specific Recommendations

IMPACT – findings on extent and condition

- **Widespread species-specific dieback of mangroves**. Severe dieback of mangroves, notably based on the common mangrove species, *A. marina*, occurred in at least 5 estuaries in the Mackay region from Sandringham Bay to Reliance and Leila Creeks.

- **All mangrove areas in the Pioneer River estuary were affected by severe and moderate dieback** in, including Basset Basin and Fursden Creek, in September 2002.

- **Most mangrove dieback areas involved *A. marina*** as either a dominant or minor component of total mangrove forest composition.

- **Mangrove dieback in the vicinity of agricultural drains was severe**. Current mangrove condition was characterised by either greater portions of dead and unhealthy trees of *A. marina*, their absence, or as growth deformities of breathing roots.

- **Mangrove dieback commenced possibly in the early to mid 1990's in the Pioneer River** notably in aerial photographs of upstream areas around Fursden Creek post 1993.

RECOMMENDATIONS regards Impact Investigations

Map mangrove dieback annually in Mackay region until there are clear indications of recovery.

Monitor mangrove health and dieback annually in Mackay region, particularly for *A. marina*, along set transects and for selected trees in affected estuaries.

Retrospective mapping of mangrove dieback to fully determine the onset and progress of dieback since 1990.

CAUSE – findings on likely agents, correlates and effects

- **Relatively high residue concentrations of herbicide diuron in mangrove sediments (~6-8 µg/kg) and core water (~12-14 ng/L)** of 3 estuaries in Mackay region, McCreadys Creek, Pioneer River and Bakers Creek.

- **Mangrove mature canopy health in field plots correlated with herbicide in sediments** where higher diuron concentrations occurred in sediments with fewer healthy trees of *A. marina* measured using leaf chlorophyll concentrations.

- **Mangrove seedling health in field plots correlated with herbicide in sediments** where higher diuron concentrations occurred in sediments with fewer healthy seedlings of *A. marina*, measured using the proportions of healthy seedlings.

- **Mangrove canopy and tree health not correlated with other potential agents** including sediment condition/burial (indicated by pneumatophore height of *A. marina*, ~5-15 cm above ground), heavy metals (including, Pb, Hg, Mn, Cu, Cd), or nutrients (including N and P).

- **Herbicide concentrations, particularly diuron, were highest at upstream water sites** and in samples collected from agricultural drains entering mangrove areas of Pioneer River (up to 1100 ng/L) and Bakers Creek (up to 900 ng/L).

- **Herbicide concentrations were unchanged from 2000** noting diuron sediment concentrations were the same in plots re-sampled in 2002 in Barnes Creek, Pioneer River.

- **Mangroves affected by herbicides (diuron, ametryn and atrazine) in planthouse trials**, noting affects on seedlings with: leaf chlorosis, necrosis and premature abscission; loss of photosynthetic function; wilting; and death.

- ***Avicennia marina* more sensitive to herbicides than other mangrove species tested in planthouse trials**, with salt-excreting species (*A. marina* > *A. corniculatum*) affected more by herbicides than salt excluders (*R. stylosa* > *C. australis*).

- **Diuron was the most toxic herbicide tested**. Herbicides were ranked by toxicity to mangrove seedlings in planthouse trials from most to least toxic: diuron > ametryn > atrazine, due in part to the relatively rapid breakdown of atrazine.

- **Herbicide levels in Johnstone and Daintree River mangrove sediments in 2002 correlated with upstream distributions of *A. marina***, noting *A. marina* was absent where diuron concentrations exceeded 2 µg/kg, and *A. marina* was present and healthy in both river estuaries where diuron concentration was low (1.5 µg/kg).

- **Diuron concentrations in mangrove sediments were 6-8 times higher where *A. marina* was present in Mackay region compared with river systems with similar catchment use.** Comparably high concentrations were found in Johnstone River mangroves where *A. marina* was absent, possibly dying many years earlier.

RECOMMENDATIONS regards Cause Investigations

Review and reassess herbicide usage, particularly diuron in Mackay region - principally to reduce loss of toxic chemicals from farms. Annual monitoring of the effectiveness of management actions in reducing levels of chemicals in mangrove sediments downstream.

Sample mangrove sediments annually in Mackay region for presence of toxic chemicals including herbicides in mangroves from Sandringham Bay to Constance Creek - noting patchiness, tidal elevation, sediment depth and sediment type.

Dose response toxicity trials on key mangrove species to determine effects at concentrations measured in the field.

Regional assessment of toxic chemicals in mangrove sediments for all river estuaries in eastern Queensland, focussing in particular on mangrove condition and toxicant presence in estuaries of high use catchments.

Retrospective assessment of possible earlier presence of *A. marina* in upstream mangrove areas of the Johnstone River.

IMPLICATIONS – findings on monitoring and mitigation

- **The severity and extent of dieback had increased from 2000 to 2002.** Mangrove health deteriorated further in Barnes Creek, Pioneer River, where the proportion of healthy trees was lower in mangrove plots re-sampled.

- **There had been a number of apparent hydrological changes as a result of the dieback.** Erosion and deposition accelerated in dieback areas, noted as erosion from higher intertidal areas and deposition into lower intertidal areas including stream channels.

RECOMMENDATIONS regards Implications and Mitigation

Investigate implications both locally and regionally - noting effects on associated biota and neighbouring marine habitats.

Assess mangrove recovery, recruitment and possible rehabilitation.

Adopt an adaptive management strategy to deal with this and future incidents.

Monitor sediment profiles from Highest Astronomical Tide (HAT) to Mean Sea Level (MSL) at least to determine the extent and amount of erosion and deposition in the dieback areas.

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It must be stated that the authors alone are responsible for the opinions expressed in this report.

Mangrove Dieback in the Mackay Region

Introduction and Project Objectives

Background

The occurrence of widespread mangrove dieback in the Mackay region of Queensland Australia was described as 'the worst of its kind in the world' (Duke *et al*., 2001). All findings and observations made since confirm that view (also noting, Kirkwood and Dowling, 2002) and there is little doubt the dieback is serious and occurs over a wide area. The consequences of such massive deterioration of coastal estuarine habitats are expected to be far-reaching and likely to affect adjacent marine habitats to the GBR if it were to worsen and not recover.

This distinctive and rare kind of mangrove dieback was severe and species-specific, affecting more than 50 km^2 of mangroves in 5 adjacent estuarine systems spread along 30 km of coastline centred around the Pioneer River estuary. It was estimated that around 58% of mangrove areas observed were affected by moderate to severe dieback. Mangrove plants, notably *A. marina,* the common mangrove tree, showed signs of unusually poor health and stress (measured as yellowed leaves and limb loss), and dead trees were not being replaced by new seedling recruits. The situation was clearly serious in June 2000. Our investigations during 2002 provide an important update with a more detailed and targeted assessment of the impact, the cause, and some implications.

Species-Specific Dieback

The characteristic that makes this kind of dieback stand out from other kinds of dieback is its apparent affect on only *A. marina*. This species is the common mangrove tree found all around Australia, extending from northern New Zealand to East Africa and Asia, throughout Indian and Western Pacific Oceans (Tomlinson, 1986; Duke *et al*., 1998). It is normally a very tolerant species, found in most extreme climatic locations for mangroves including very wet and very dry climates from wet tropic to arid desert regions, in a broad range of salinity regimes from offshore islands to upstream locations in river estuaries, and in equatorial to temperate latitudes. In this way, its distributional range notably exceeds that of all other mangrove species in the Indo West Pacific region. The plant is also highly resilient since it normally resprouts readily from physical damage like limb and foliage loss inflicted by strong storms, or cutting (Wadsworth, 1959). And, it normally has prolific fruit production and abundant seedling establishment. It is therefore unusual to find this species as the one specifically affected by dieback. The first observation is that the agent causing this kind of dieback must be something relatively uncommon and unnatural. Furthermore, *A. marina* plants must have some uncommon characteristic that makes them more vulnerable than other mangrove species.

Subsequent investigations into the cause have focussed on finding such a characteristic. Two possible attributes of *A. marina* were investigated in 2002, including gaseous exchange through emergent breathing roots called pneumatophores, and a special physiology called salt excretion. However, while neither of these attributes are unique to *A. marina* the aim was to discover whether either makes this species more vulnerable than other species with like features. A chief goal of our 2002 investigations was to gather data concerning each attribute so we might better evaluate which, if any, might be related to the dieback observed in the Mackay region.

Only one other instance of this kind of dieback had been reported previously, noting the instance in the early 1970's around the Calliope River near Gladstone (Saenger, 1988). In this case, however, the extent was far less than that reported for the Mackay region (Duke *et al*., 2001) and the cause was not identified. Plant pathogens were suspected, but these were found to occur naturally in the

area, and it was concluded that these pathogens might act only after trees were stressed by other agents (Prof. J. Irwin, UQ, personal communication).

Justification for Investigations in 2002

The justification for further research and its direction was made largely in the preliminary report by Duke *et al*. (2001). As stated in the previous report, it was imperative to find the true cause of dieback. Such information is essential if this serious environmental problem is to be fixed, and the damaged habitat restored. For example, if herbicides were definitely implicated then the immediate mitigation action would be to reduce herbicide concentrations in mangrove sediments, and to allow the habitat to largely restore itself. In this, the benefit would be 2-fold since herbicides lost from farms in run-off is a lost expense by farmers. However, given that herbicide levels in runoff were already unacceptably high (NRM, 2002), finding ways to reduce herbicide amounts lost in run-off, might seem an immediate beneficial action to improve farm income, irrespective of other considerations.

Recommended mitigation actions were delayed by apparent uncertainty inherent in the preliminary research findings. A primary goal for studies in 2002 was to provide greater certainty in deciding which was the most likely agent, and to provide reasonable proof of its effect, if possible. The essential first step in resolving the uncertainty however was made in the preliminary report when herbicides were identified and isolated as the most likely agent after careful and thorough consideration of at least 12 possible causes. Other agents were not to be ignored in future assessments, but understandably, greater attention was to be paid to the most likely one. The investigations proposed had very limited funding so they needed to be carefully managed to maximise research outcomes while all opportunities were taken to enhance the program whenever possible.

The program proposed included one major component, an intensive field survey in the Mackay region to quantify the extent of dieback in 2002, and to compare mangrove health with a selection of possible agents in replicated sites. Possible agents or associated factors included herbicides, heavy metals, nutrients, and sediments. Another key consideration was to repeat these studies in 3 adjacent estuarine systems in order to identify any common agent between them. This was considered essential in order to test whether the likely agent correlated with dieback areas seen in aerial surveys. In this way, our investigations were directed mostly, but not exclusively, toward the relationship between herbicides and condition of mangrove trees, especially *A. marina*.

Key Objectives of the Investigation

Investigations undertaken in 2002 were based on 3 research components chosen to complement and progress our overall enquiry, and to identify the cause of dieback. These components and sub-components address a selection of key questions raised by the preliminary investigation (Duke *et al*., 2001). The success of our current investigation is based on the answers to these questions.

1) Surveys of mangrove dieback in the Mackay region.

- **Aerial surveys** to describe and quantify the extent of dieback in 2002, involving: a light aircraft survey of areas from Sandringham Bay to Reliance and Leila Creeks in May; and an aerial photographic survey of 3 estuaries, McCreadys Creek, Pioneer River and Bakers Creek in September.

***Questions**: Was the area of dieback expanding? Was A. marina dieback the more dominant kind of dieback? What was the current extent of mangrove dieback in individual estuaries, and in the region? Was it possible to establish a baseline record of dieback in 2002?*

- **Field surveys** of McCreadys Creek, Pioneer River and Bakers Creek to assess mangrove condition and health in May, and to compare the results with measurements and sampling of the most likely factors causing dieback, involving: detailed plots, rapid transects, and river water stations.

***Questions**: Was dieback getting worse? Was* A. marina *more affected by dieback than other mangrove species in estuaries north and south of the Pioneer River, including McCreadys and Bakers Creeks, respectively? Did root burial selectively affect* A. marina *trees and contribute to their death? What is the relationship between pneumatophore height and mangrove health for* A. marina*? Was there any seedling recruitment and recovery? Were herbicide residues higher in upstream water? What was the condition of* A. marina *in agricultural drains, and what herbicides were present? Had there been a change in herbicide concentrations found in plots sampled in 2000?*

2) Preliminary toxicology trials in the planthouse (Honours project by Alicia Bell)

- **Planthouse trials** with seedlings to show effects of 3 herbicides on 4 mangrove species identified in the preliminary field investigation, comparing differences between salt excluding and salt excreting species.

***Questions**: Were mangroves affected by herbicides? If so, was A. marina more sensitive than other species commonly found in Mackay region? Were salt excreting species more sensitive than salt excluding species?*

3) Comparative river studies assessing *A. marina* and herbicides (additional field projects)

- **Field surveys** to gather comparative data on presence and condition of *A. marina* mangroves and herbicide presence in sediments of other Queensland river systems influenced by cane agriculture, including Johnstone and Daintree Rivers.

***Questions**: Was species-specific dieback only occurring around Mackay? Why was the dieback only occurring around Mackay? Was A. marina found in areas where herbicide levels are as high as those found in Mackay region? Were mangroves affected by herbicides in other rivers?*

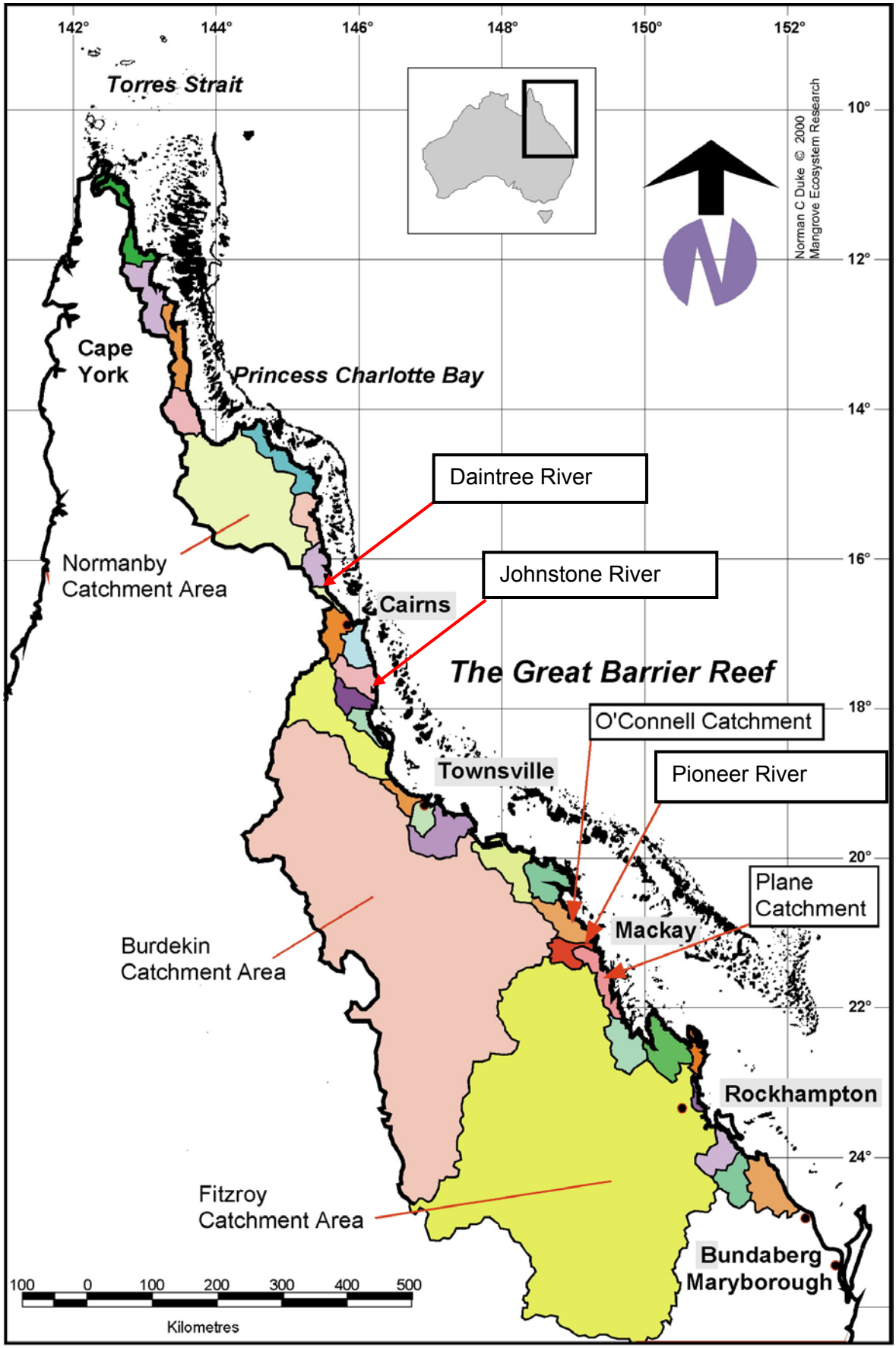

FIGURE 1: Catchment areas along the north and central coast of Queensland showing relative locations of Pioneer (plus McCreadys and Bakers Creeks), Johnstone and Daintree Rivers. These were the estuaries sampled during investigations in 2002.

Methods

Study Areas

The main study areas were located within 4 main estuaries situated between 20^{o} and 22^{o} south (Figures 2 and 3). These were Eimeo and Bucasia, McCreadys Creek, Pioneer River (Barnes Creek and Fursden Creek) and Bakers Creek. Eimeo and Bucasia were considered to be relatively unaffected by the mangrove dieback, while the other three areas were identified as the areas where the mangrove dieback was occurring.

Eimeo and Bucasia Creeks

The estuaries of Eimeo and Bucasia Creeks (Appendix 1 and 2 respectively) are the most northerly-situated study areas in this investigation. Compared with all other river/creek systems in the main study area, these are the least anthropogenically impacted.

None of the mangroves in Eimeo and Bucasia Creek estuaries were affected by dieback in 2000 (Duke *et al*., 2001). There were no study sites in Eimeo or Bucasia Creeks in the current investigation, however these creeks were mapped in the aerial investigation.

McCreadys Creek

McCreadys Creek estuary (Appendix 3) is located north of the Pioneer River (Figure 3) and discharges onto the intertidal flats immediately north of the suburbs of North Mackay and Andergrove. The physical condition of McCreadys Creek is relatively intact, albeit dominated by urban development in the south and a golf course and cane farms to the west and north. The estuary would be affected mostly by the runoff from these areas.

Approximately 22% of the mangroves in McCreadys Creek estuary were affected by dieback in 2000 (Duke *et al*., 2001). The study sites used in this survey are shown in Figure 4.

Pioneer River

The Pioneer River estuary (Appendix 4) is situated between latitudes 21^{o} and 21^{o}25' S and extends approximately 16.5 km from the tidal limit at Dumbleton Rocks, to the mouth. The Pioneer River catchment is a relatively small (1570km^2), geomorphologically dynamic catchment (Arthington *et al*., 2001). Natural processes, including channel migration, sand splay deposition on the floodplain and river mouth migration have been modified to some degree by human intervention (Arthington *et al*., 2001). As a result of the steepness of the upper catchment, water flow within the Pioneer River is generally fast, with associated high turbidity levels (NRM, 2002). Runoff is variable, with over 80% of the annual discharges occurring in flood flows during cyclone season from December to April (PICMA, 1995). Most deposition and flooding occur in and around the estuary (Gourlay and Hacker, 1986). Land use in the Pioneer River catchment is dominated by agricultural industries. Approximately 296km^2 of the catchment is allocated to sugarcane production predominantly on the river flats (GBRMPA, 2001a). State forest and timber reserves cover an area of 354 km^2, and the majority of the rest of the catchment is used for cattle grazing which occupies about 1166km^2 (GBRMPA, 2001a).

A large portion of the severe mangrove dieback is located in Bassett Basin within the Pioneer River estuary. (Duke *et al*., 2001). The Bassett Basin is a large body of water protected by waves and wind by coastal dunes and is isolated from the main river channel as normally only tidal creeks, including Barnes, Gooseponds and Vines Creek, drain into it (Gourlay and Hacker, 1986). The

physical structure of the Basset Basin has changed significantly from the natural conditions, primarily due to the impact of training walls on hydrodynamics and sediment transport processes (Arthington *et al*., 2001). The initial construction of the north wall across the southern margin of Bassett Basin between 1887 and 1892 caused restriction of tidal circulation and accelerated sediment accretion in the area (bought down Barnes and Vines Creeks during times of flood flows as well as material brought in by tidal action) (Gourlay and Hacker, 1986) and subsequently, the eastern portion of the wall was lowered in 1903. Low tide levels in the Bassett Basin are held up by the training wall at the entrance to the Bassett Basin (Arthington *et al*., 2001), which would probably lead to impeded drainage. During medium and major floods the main part of the Pioneer River overflows into Barnes Creek.

In 1992, a report on the status of stream banks in the Pioneer River indicated that the mangrove vegetation of Bassett Basin was in 'very good condition', however along other parts of the river, there were few parts found to be without some form of degradation (Hill and Hunt, 1992). Another study on the vegetation communities in the Mackay Region in 1995 did not detect the current dieback (Winter and Wild, 1995), which is believed to have started between 1995-1997 after a number of major runoff events (Appendix 20). Aerial photographs of Fursden Creek (Pioneer River estuary) in 1997 and 2002 show large canopy patches not previously seen in a 1993 aerial photograph - see Appendix 7, 8 and 6 respectively. Aerial photographs from 1993 and 2002 at the STP creek are also presented in Appendix 9 and 10 respectively. The 1993 photos do not show dieback of *A. marina*. Aerial oblique photographs dated at May 25, 1998 from the STP creek in Bassett Creek in Bassett Basin show evidence of advanced dieback, with large *A. marina* along the creek as dead or unhealthy (sick) trees (Figure 47).

Before the dieback, the mangrove vegetation in the Pioneer estuary had been observed to increase in extent and density in certain areas (Gourlay and Hacker, 1986). The mangrove communities in the Bassett Basin were well developed as a result of reduction in salinity in high intertidal areas due to increased flooding frequency, higher rainfall, an increase in nutrient supply to the estuary and increased sedimentation (Gourlay and Hacker, 1986). Mangrove loss however is also quite significant. Winter and Wild (1995) compared historical records of mangroves in 1953 to 1993 and identified a loss of mangrove area by 30%. This loss averages around 5 ha per year (Duke *et al*., 2001) and may be attributed to the progressive encroachment and replacement of tidal lands with landfill to create large-scale developments such as the harbour, shopping centres and urban subdivisions.

At least 86% of the mangrove forests in the Pioneer River estuary were affected by dieback in 2000 (Duke *et al*., 2001). Study site locations are shown in Figure 5.

Bakers Creek

Bakers Creek estuary (Appendix 5) discharges onto the intertidal flats south of the Pioneer River (Figure 3). The physical condition of Bakers Creek is largely relatively intact, however there have been a number of human-related activities that have affected the physical condition of Bakers Creek estuary. Direct activities include extraction of sand from the estuary for dredging and associated drainage works linked with the construction of the airport, and historical bank stabilisation and river training works through construction of bund walls (Winter and Wild, 1995). Indirect human-related activities include urban runoff inputs, including from Plainlands and Paget in Mackay, and runoff from cane farms. In Bakers Creek, sugar cane faming and grazing are the dominant land uses. There is increasing development of aquacultural practices for prawn and red claw farming (Winter and Wild, 1995).

There are extensive mangrove wetlands in the lower reaches of the river, below the Bruce Highway. Since 1953, 18 hectares (4%) of mangroves and 45 hectares (22%) of saltmarsh/saltpan communities have been lost, particularly upstream of the Bruce Highway (Winter and Wild, 1995).

A. marina dieback has also been documented in Bakers Creek estuary. Approximately 38% of mangroves were affected by dieback in Bakers Creek estuary in the preliminary investigation (Duke *et al*., 2001). The study sites in Bakers Creek in this investigation are shown in Figure 6.

FIGURE 2: Satellite image of the Mackay region, encompassing the study areas of Eimeo Creek, Bucasia Creek, McCreadys Creek, Pioneer River and Bakers Creek.

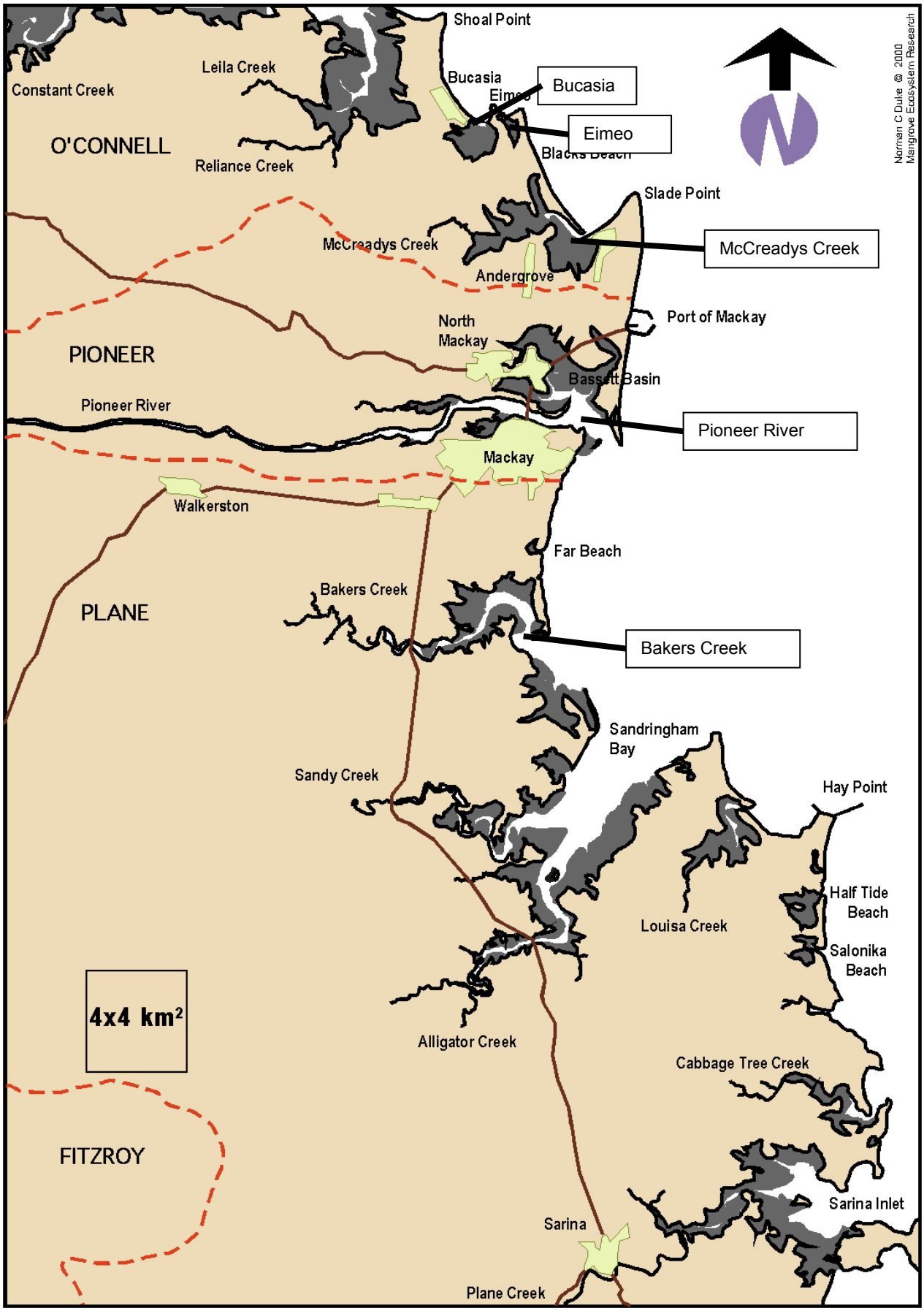

FIGURE 3: Map of the Mackay region showing the six estuaries surveyed in the aerial overflight, including Leila/Reliance Creeks, Bucasia/Eimeo Creeks, McCreadys Creek, Pioneer River, Bakers Creek and Sandringham Bay –Sandy/Alligator Creeks.

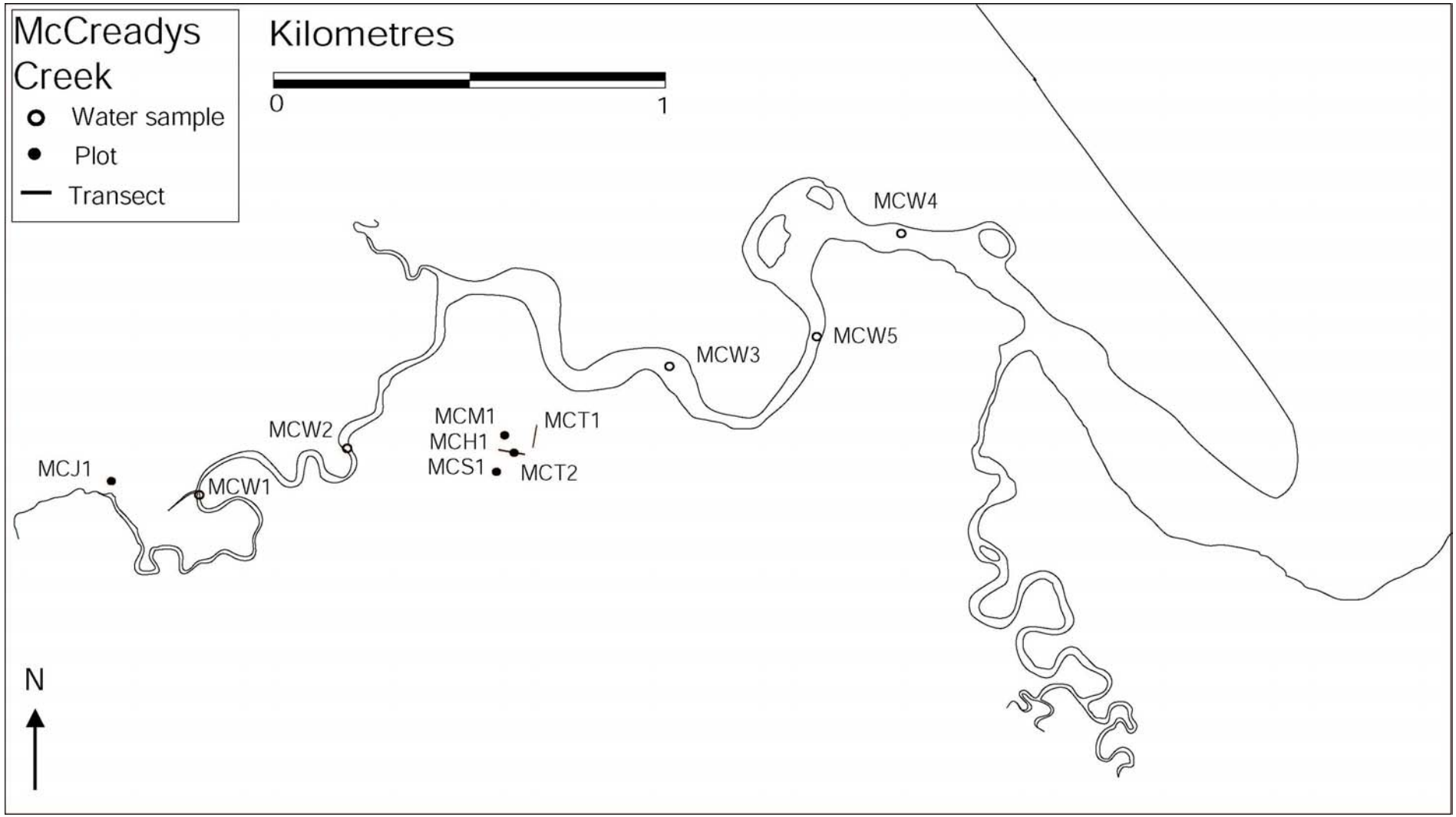

FIGURE 4: McCreadys Creek estuary study sites showing water sampling, plot and transect locations.

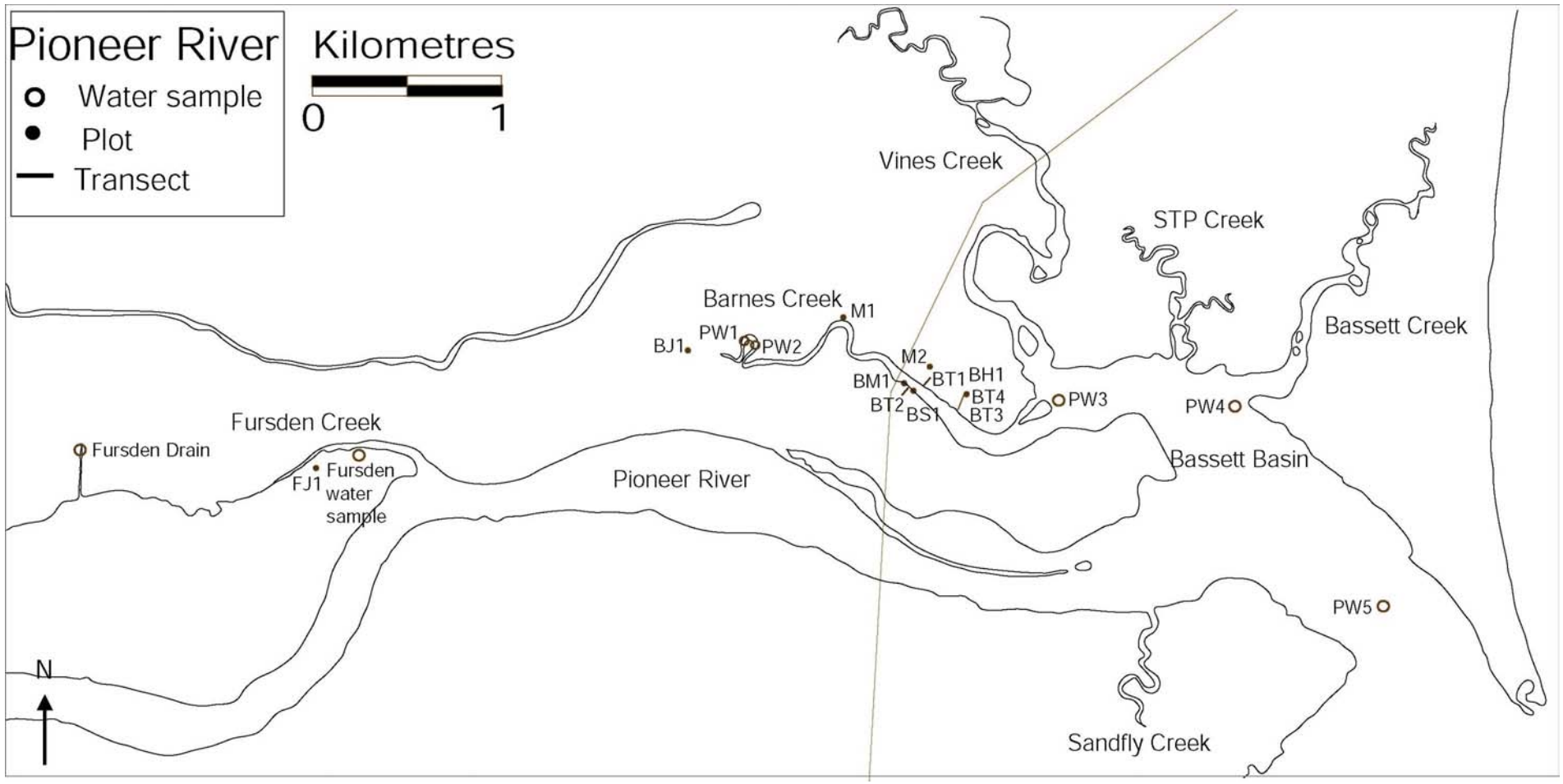

FIGURE 5: Pioneer River estuary study sites showing water sampling, plot and transect locations.

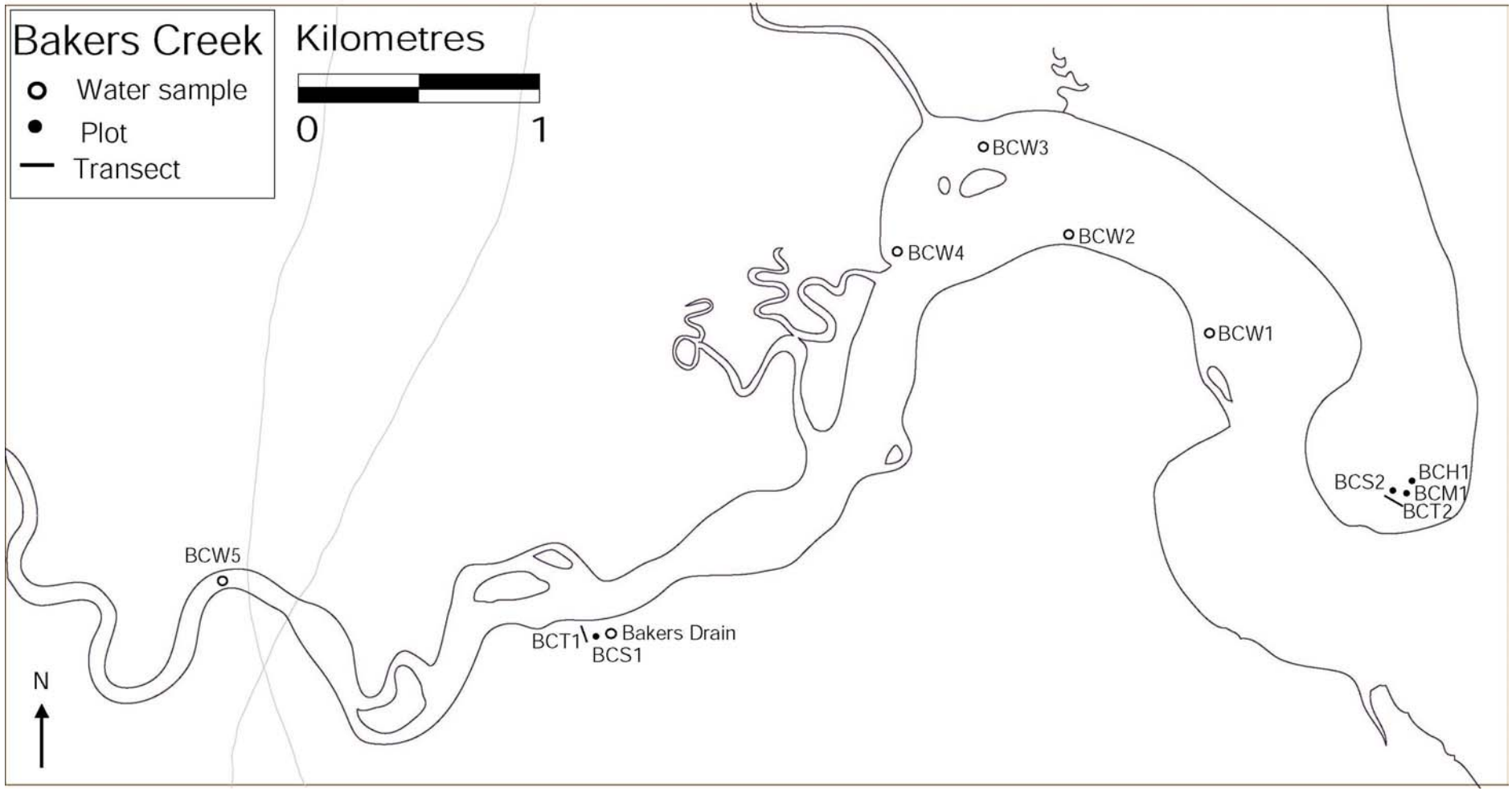

FIGURE 6: Bakers Creek estuary study sites showing water sampling, plot and transect locations.

Extent of Dieback and Mangrove Condition

Light Aircraft Survey

An aerial flight was conducted on 1 May, 2002 over the broader study area to observe the condition of dieback and general mangrove condition, and obtain photographic imagery to compare current levels of dieback with that observed during the overflight in 2000. Three observers took records of the flight path and general observations of dieback, and oblique still and video imagery. Surveyed areas were within 15-20 km north and south of the Pioneer River estuary. All mangroves in the Pioneer catchment estuary were viewed including those along the river, upstream in Fursden Creek, and those bordering Bassett Basin. The O'Connell catchment area was surveyed in part, including the three larger tidal areas immediately north of the Pioneer River estuary: Reliance/Leila Creeks, Bucasia/Eimeo Creeks and McCreadys Creek. The Plane catchment area was also surveyed in part, including the two larger tidal areas immediately south of the Pioneer River estuary: Bakers Creek and Sandringham Bay - Sandy/Alligator Creeks.

Aerial Flight and Image Acquisition

Aerial photographs were acquired for the Mackay Region, from Alligator Creek, to the Northern Beaches (Eimeo and Bucasia) by Australasian Mapping Services, Brisbane, Queensland. Photographs were taken at 5000ft during September 2002. The photographs were scanned at 600 dots per inch (dpi) and were then resampled with *Adobe Photoshop* (version 6.0) to reduce file size from 60 to ~10 megabytes. A mosaic was created using Adobe Photoshop (version 6.0) of areas of interest. The mosaic was georeferenced using *ERDAS Imagine* (version 8.5) to a LANDSAT ETM 5 image of the regions, which was acquired in 1995 by the Biophysical Remote Sensing Group, Geophysical Sciences (The University of Queensland). Approximately 20 points were used to georeference the digital mosaic. The mosaic was imported into GIS ArcView 3.2, where vegetation type and health boundaries were digitised. This resulted in polygons, which were labelled accordingly. Using GIS tools, surface areas were calculated and several maps produced. In addition to using digitised images as a means of vegetation classification, a stereo-viewer was also used to aid interpretation.

Vegetation Types

Mangrove - The spatial extent of mangroves in the estuary was calculated by creating polygons around all identified areas. Saltmarsh/saltpan areas were not included in this investigation.

'Mixed' *A. marina* - The spatial extent of *A. marina* was delineated based on their recognisable canopies of healthy, sick and dead trees. Both were included in polygons. Furthermore, as *A. marina* trees rarely occur as a continuous canopy in the region, the classification usually included a mixture of species (eg. Figure 10E).

Dieback Classification

Health and condition of mangrove canopies were classed in 3 categories based on presence of obvious dieback as None, Moderate and Severe (Table 1).

TABLE 1: Description of vegetation classes used for this assessment of mangrove dieback were based on those used in the preliminary investigation (Duke *et al*., 2001).

Dieback classes	Description of Mangrove Forest Condition
None (unaffected)	Low level dieback, less than 15% overall
Moderate	Greater than 15% and less than 30% of mangroves found as sick and dead trees
Severe	Numerous sick and dead trees. Greater than 30% of mangroves found as unhealthy and dead trees

Ground Survey - Mangrove Condition and Assessment

Mangrove Plots

Plots were selected on the basis of *A. marina* health only. A minimum number of 3 plots were chosen for each estuary, which were None (unaffected), Moderate and Severe (Table 1). There were 5 mangrove plots in the Pioneer River estuary (all in the Barnes Creek area), 3 plots in McCreadys Creek and 4 plots in Bakers Creek. It was not possible to find a plot without any mangrove dieback. Plot sizes ranged from $16m^2$ to 100 m^2. All data was collected between 29 April – 3 May, 2002. The Department of Primary Industries and the Mackay community collected data on stand and seedling structure on further occasions at Eimeo (1 August, 2002), Barnes Creek (9 August 2002), Fursden Creek (with pneumatophore height data) (19 July, 2002) and McCreadys Creek (19 September 2002), using methods as detailed in the following sections.

In order to determine stand structure, species identification, height (estimation where not possible to measure) and circumference of all trees in the plot were measured. Seedling demography was measured in the same plot as the stand structure measurements (seedlings were identified as mangrove trees <0.5m tall). The following parameters were recorded; node count, height, circumference and leaf number. Often the plot size was reduced, as the number of seedlings within each plot was too large.

Health of individual mangrove trees (of all species) was classed into 3 categories of health, being - healthy, sick or dead (Table 2)

TABLE 2: Classification and characteristics of condition and health mangrove trees used in this investigation.

Classification	Characteristics
Healthy	Leaves green, no visible signs of sickness
Sick	Yellowing, wilting leaves Low foliage cover
Dead	Plant dead

Plots were then classified as having no dieback, moderate or severe dieback based on the same data as in Table 1. These classifications were made on *A. marina* trees only within the plot. Seedlings were not incorporated into this classification for a number of reasons. Seedlings and trees are potentially affected by toxicants differently. If toxicants are patchy in the sediment, seedlings within a small plot will be affected differently depending on their situation with regards to the presence of the toxicant. Seedlings also have their roots in a shallower part of the sediment profile to trees. Considering that this report is specifically looking at potential toxicants, it is not suitable to assess mangrove health on the basis of both trees and seedlings. Furthermore, trees and seedlings have different physiologies, for example, *A. marina* trees have pneumatophores while seedlings do not. Finally, there are other natural factors that affect seedling health that do not affect mangrove trees, such as the effect of shading. If seedlings are unhealthy or absent underneath a canopy of mangrove trees, it will not necessarily mean that the plot is unhealthy.

Photosynthetic Efficiency - A PAM (Pulse Amplitude Modulated) fluorometer (Walz, Germany) was used to determine the photosynthetic efficiency of the leaves of each species of mangrove in each plot. At least 3 readings were taken for each species in the plot. Species that were not within the plot but were in close vicinity to the plot were also measured. This was done by placing dark-adapting leaf clips on an attached leaf from the second leaf pair on the branch. Leaves were left to dark-adapt for at least 15 minutes. The fluorescence signal was measured from the mid-portion of the adaxial leaf surface, away from the mid-vein and any damaged parts of the leaf. After this time, the PAM was used to get values for F_v/F_m, F_o (Initial Fluorescence) and F_m (maximum fluorescence). Measurements were made throughout the day.

Rapid Transects
Three rapid transects were conducted at each of the dieback sites to ascertain the nature of the dieback (i.e. to assess if *A. marina* was the only mangrove species dying). These belt transects were four metres wide and ran in most cases from the landward margin to the river edge of mangrove extent. Where this was not possible, transects were run for approximately 50m. Every five metres, a 4x5m plot was set up and data collected. A number of parameters were measured in these plots including those indicating forest structure (tree height, alive/dead, canopy condition, number and height of pneumatophores, number of species, 'canopy health'), sediment condition (i.e. evidence for sediment erosion/deposition) and core water samples for LCMS analysis. Transect data on forest structure collected in these surveys were used as ground truth for the mapping of dieback in the Mackay region.

Ground Survey - Erosion and Sedimentation

Pneumatophore Depth
Twenty living *A. marina* pneumatophores per plot were chosen randomly, and measured from the tip of the pneumatophore to the sediment, and from the sediment surface to the cable root. These numbers were used to determine whether there was a difference in pneumatophore height indicating burial or erosion between healthy and non-healthy sites.

Stakes
It was important to monitor long-term changes in sediment accretion/erosion in the area; therefore a number of stakes were positioned at the plot locations in the Mackay estuaries. Wooden stakes were firmly pushed into the sediment in each plot and location of the stakes was recorded with a GPS. Stakes were marked with a site code, date and height from the sediment to the top of the stake. These measurements were also recorded on data sheets. This was done so that further monitoring of sedimentation or erosion could take place.

Sample Collection and Analysis

All samples were collected using gloves to prevent contamination from skin. Containers for herbicide and metal sampling in water and sediment, and nutrients in water were quality-controlled bottles, obtained from QHSS (Queensland Health Scientific Services).

Plot/ Drain sampling

Sediment
Samples were collected for determination of organic matter, benthic microalgae, herbicides and heavy metals.

Organic Matter in Sediment - Sediment was collected and placed in a clean plastic ziplock bag. Samples were frozen ASAP. Organic matter in soil can be estimated with reasonable accuracy by the weight loss on ignition method (Schulte and Hopkins, 1996). In the lab, samples were dried in a drying oven at 60^{o}C for 24 hours. The dried sample was weighed on a balance with dessication beads to prevent weight gain from moisture absorption by the soil. After weighing, the sample was placed in a combustion oven to burn the organic matter for 3 hours at 550^{o}C. Combustion at 550°C converts organic matter to CO_2. Samples were removed from the combustion oven and put into the drying oven for a further 24 hours. The sample was weighed again on a balance with dessication beads. Percentage organic matter of the sediment was quantified as proportion of weight lost after combustion, however the main limitation to this method is that the weight loss on ignition procedure tends to overestimates the organic matter content of soils because it includes loss of other volatiles H_2O, structural OH, CO_2 from carbonates) (Schulte and Hopkins, 1996). In spite of this, the

sensitivity for this method is reported to approximately 0.2 to 0.5% organic matter, unless soils are highly calcareous (Soil and Plant Analysis Council, 2000).

Benthic Microalgae (BMA) - 5 sediment cores for BMA were sampled randomly in each plot from undisturbed sediments with a cleaned cut-off 60mL syringe, to 2 cm depths. The sediment cores were transferred into clean 50mL centrifuge tubes, kept in the dark and cold in the field and frozen as soon as possible. In the lab, samples were ground with 90% acetone, 10% distilled water and made up to 40mL with acetone. Samples were stored in the freezer (-16^{o}C) to allow for pigment extraction for 24 hours and shaken once during this period. Samples were centrifuged for 20 minutes at 2000 rpm. 1 mL of solution was transferred using a glass pipette to a glass cuvette (1cm path length), and wavelengths were determined on a Pharmica LKB Ultrospec III Spectrophotometer. Wavelengths chosen for analysis were 647 and 664nm for the chlorophylls. BMA concentrations were calculated in mg m^{2-1} using methods from Jeffrey and Humphrey (1975) and Lorenzen (1967).

Sediment Herbicides - Samples of sediment for herbicide analysis were collected in a 375mL solvent washed glass container – mixture of 5 sub-samples over the plot. These samples were taken from surface sediment, ie. to depths of ~5 cm. Samples were kept cold and frozen as soon as possible. Sediment samples were transported frozen and were analysed at QHSS for a wide range of herbicides known to be used in the Pioneer River Catchment. These were diuron, atrazine, ametryn, simazine, tebuthiuron, fluometuron, hexazinone and prometryn. Solvent (acetone/hexane) was added to the sediment and shaken overnight on a mechanical shaker to allow for physical separation of bulk water. The extract was then filtered through anhydrous sodium sulphate to remove residual water. This extract was then concentrated by rotary/vacuum evaporation and the solvent was exchanged to methanol/water for LC/MS/MS determination against prepared standards of the herbicides requested. A separate sediment sub-sample was used to determine dry weight for calculation. The results were expressed in micrograms per kilogram (µg/kg) on a dry mass basis.

Sediment Heavy Metals – Samples of sediment for heavy metal analysis were collected in a 375mL acid washed glass container from a mixture of 5 sub-samples over the plot. These samples were taken from surface sediment, ie. to depths of ~5 cm. Samples were kept cold in the field and frozen as soon as possible. Samples were transported frozen and were analysed at QHSS for the following heavy metals: Arsenic (As), Chromium (Cr), Copper (Cu), Iron (Fe), Manganese (Mn), Nickel (Ni), Lead (Pb), Zinc (Zn), Mercury (Hg) and Cadmium (Cd). Sediment samples were prepared to AS4479 and then crushed in a zirconia tema swing mill. Analyses were conducted on the finely ground powders. As, Cu, Fe, Mn, Ni and Pb were analysed by acid digestion (USEPA3050A). Zn, K, Ca, Mg and Na were analysed by inductively coupled plasma atomic emission spectrometry (MGM-017). Cd was analysed by flame atomic absorption spectrometry (MGM-024), Hg by cold vapour atomic absorption spectrometry (MGM-021) and Cl by X-ray fluorescence spectrometry (1924). The analysis was carried out on the finely crushed sample. Results were reported in milligrams per kilogram (mg/kg) on a dry mass basis.

Core Water

In each plot, 5 holes were dug in the mangrove sediment during low tide approximately 30x30cm until there was sufficient water in the hole for sampling, usually ~30cm deep. Core water holes were taken from root depth in the sediment. From the core water in each hole, samples for pesticides, nutrients (filtered and unfiltered) and heavy metals were collected. The physical properties (ph, redox, temperature (^{o}C), salinity (‰) and dissolved oxygen) of the core water were also measured.

Pesticides - A 1L solvent washed glass jar was filled with water from each of the 5 holes in the plot. A 60mL sterile syringe was used to collect the water. Samples were kept cold in the field and refrigerated ASAP. The water samples were first defrosted and then filtered prior to extraction. A glass microfibre filter (Whatman GFA 90 mm ∅) was rinsed with acetone then thoroughly rinsed with deionised water before being clamped in place over a scinter under a funnel. A water sample

was added to the funnel and collected in a modified Schott bottle. The filtered water samples, as well as a control sample consisting of 1 L of RO water, were extracted by solid phase extraction (SPE) using an Oasis Extraction Cartridge, (Waters, HLB 12cc 500mg LP). SPE cartridges were conditioned with 5mL of methanol followed by 5mL of deionised water. The cartridge was fitted into a lid made for the Schott bottle with a seal. The Schott bottle was then inverted; a valve was opened in the bottom of the bottle and the water sample passed slowly through the cartridge. After the sample had passed through the cartridge, the Schott bottle was rinsed with 100mL of RO water, which was also extracted through the cartridge. Following the extraction of the water on to the solid phase, the cartridges were eluted with 10mL of methanol. One millilitre of methanol was first eluted through the cartridge, then discarded, to remove water held in the cartridge. The cartridges were fixed in place over clean 15mL calibrated test tubes and 10mL of methanol was added. Once the 10mL had eluted through, the sample was spiked with 50μL of dimethylsulfoxide (DMSO) as a keeper and were then reduced using a gentle stream of nitrogen to 0.5mL. The sample was then made up to exactly 1mL with deionised water and transferred to vials ready for analysis by LCMS. The samples were analysed for 8 herbicides; tebuthiuron, hexazinone, ametryn, prometryn, atrazine, diuron, fluometuron and simazine. Results were expressed in nanograms per litre (ng/L).

Heavy Metals - A 250 mL acid-washed plastic container was filled with water from each of the 5 holes using a 60mL sterile syringe to sample approximately 45mL from each hole. Nitric Acid (2.5mL 70% v/v) was added to the water sample in the bottle. Samples were kept cold in the field, and frozen as soon as possible. Core water samples were analysed at QHSS for the following heavy metals: Arsenic (As), Chromium (Cr), Copper (Cu), Iron (Fe), Manganese (Mn), Nickel (Ni), Lead (Pb), Zinc (Zn), Mercury (Hg) and Cadmium (Cd). Analysis was carried out on the water samples using method MGM-018. Results were expressed in micrograms per litre (µg/L).

Nutrients –

Soluble (filtered) water samples were collected in 100mL Reverse Osmosis Water washed plastic bottles. A 60mL sterile TERUMO disposable syringe was used to collect the water sample, which was filtered using a SARTORIUS "Minisart" hermetically sealed filter with 0.45µm pore size. The syringe was rinsed twice with sample water and filled completely with sample water (approximately 60mL) and filter was attached. Approximately 10mL of sample was filtered in to the sample bottle, bottle was capped, shaken and sample discarded, repeated. The remaining 40 mL of sample was filtered into the bottle. Filters were replaced when necessary. Bottle was capped, leaving 2cm airspace. Samples were kept cold in the field and frozen when possible. Samples were analysed at QHSS for Phosphorus (total dissolved and filterable reactive phosphorus) and Nitrogen (ammonia, oxides and total dissolved nitrogen) in accordance with the methods of Clesceri *et al.* (1998) using a Skalar autoanalyser (Norcross, Georgia, U.S.A.).

Total (unfiltered) water samples were collected with 60mL TERUMO sterile syringes in 250mL Reverse Osmosis Water washed plastic bottles. Samples were kept cold in the field and frozen when possible (within 12 hours). Samples were analysed at QHSS for Total N and Total P in accordance with the methods of Clesceri *et al.* (1998) and Hosomi and Sudo (1986) using a Skalar autoanalyser (Norcross, Georgia, U.S.A.).

Physical Properties - The sediment core water was tested for pH, dissolved oxygen (% saturation), redox potential (mV) and temperature (^{o}C) using a TPS 90-FL instrument at each site. The TPS field instrument was calibrated according to the TPS 90-FL instruction manual. Data was collected by inserting the probes into the sediment core water, and taking the reading when it had stabilised. Salinity was measured with a portable refractometer by adding a few drops of water onto the lens, and obtaining the reading through the adjustable eyepiece. Instruments were cleaned between sample sites to prevent cross contamination of the samples. The range(s), resolution and accuracy of each measurement are displayed in Table 3.

TABLE 3: Range, resolution and accuracy of TPS 90-FL field instrument with measurements of pH, millivolts, dissolved oxygen (DO) and temperature, and salinity using a portable refractometer.

	Range(s)	Resolution	Accuracy
pH	0-14pH	0.01	±0.01
Millivolts	0- ±1999mV	1mV	±1mV
DO	0-300% sat	0.1% sat	±0.2% sat
Temperature	-10.0-110^{o}C	0.1^{o}C	±0.2^{o}C
Salinity	0-100ppt	1ppt	±1ppt

ToxY-PAM - Like all PAM fluorometers, the toxY-PAM applies pulse modulated measuring light to assess the yield from chlorophyll fluorescence. All methods for using toxY-PAM were according to Schreiber *et al*. (2002), using a *Phaeodactylum tricornutum* algal culture.

Vegetation

Vegetation was collected of mangrove species in each plot, in particular *A. marina.* A sufficient amount of healthy and sick leaves, as well as twigs from dead trees were collected from mangroves within each plot and put into clean plastic ziplock bags for sample analysis. Leaves collected were fully expanded leaves from the second leaf pair on the branch, without notable damage. Samples were washed thoroughly in distilled water to remove possible contaminants from the surface of the vegetation, and frozen. These samples were analysed for δ15N, %N and Carbon 13, chlorophyll content, herbicides and major elements.

Leaf Nutrients - Leaf samples were dried at 60^{o}C prior to grinding and analysis. Samples were analysed for δ^{15}N, elemental % nitrogen (%N) and δ^{13}C using a Micromass continuous flow stable isotope ratio mass spectrometer (Diocares, R., Griffith University).

Leaf Chlorophyll - Leaf samples were weighed and ground in a small amount of 90% acetone using a mortar and pestle. The solution was then poured into a 10mL centrifuge tube and made up to 10mL with acetone. Samples were stored in the freezer (-16^{o}C) to allow for pigment extraction for 24 hours and shaken once during this period. Samples were centrifuged for 20 minutes at 2000 rpm. 1 mL of solution was transferred using a glass pipette to a glass cuvette (1cm path length), and wavelengths were determined on a Pharmica LKB Ultrospec III Spectrophotometer. Wavelengths chosen for analysis were 647, 664, 610, 510, 480 nm. Chlorophyll analyses included determination of chlorophyll *a*, chlorophyll *b*, carotenoids, total chlorophyll (*a*+*b*) and ratio of chlorophyll *a*:*b*. Acidification with 10% HCl was used to correct analysis of chlorophyll a from interference from Mg-free chlorophyll derivatives (phaeophytins and phaeophorbides, collectively termed 'phaeopigments') (Jeffrey, 1997).

Leaf herbicides - Leaf samples were analysed at QHSS. Samples were analysed for the herbicides diuron, atrazine, ametryn, simazine, tebuthiuron, fluometuron, hexazinone and prometryn. The vegetation was blended at high speed with acetone. Dichloromethane was added to physically separate bulk water. The extract was then filtered through anhydrous sodium sulphate to remove residual water. This extract was then concentrated by rotary/vacuum evaporation and the solvent was exchanged to methanol/water fro LC/MS/MS determination against prepared standards of the herbicides requested. Results were expressed in micrograms per kilogram (µg/kg) on a fresh weight basis.

Leaf major elements - Leaf samples were analysed at QHSS. Leaf samples were dried at 50^{o}C overnight and then crushed in a tema swing mill. Analyses were carried out on the finely crushed sample. Samples were analysed for Potassium (K), Sodium (Na), Calcium (Ca), Chromium (Cr), Copper (Cu), Iron (Fe), Manganese (Mn), Magnesium (Mg), Zinc (Zn) and Chloride (Cl).

Water Column Sampling

At each estuary there were five water sampling sites ranging from upstream to downstream locations. Samples were collected on the same days as the plot sampling and data collection. Data was collected at high tides on each of the days.

Chlorophyll - Samples were collected for chlorophyll in the water column in 2 litre plastic bottles. Samples bottles were rinsed three times before water collection and water was collected as a sub-surface grab sample (approximately 20cm below the water surface to avoid surface scum). Samples were kept cold in the field and then filtered through a 0.45µm Whatman GF/C glass microfibre filter paper using a pump and filter towers. The filter papers were wrapped in aluminium foil and frozen. In the lab, the frozen filter paper was ground using a mortar and pestle and made up to 10mL in a centrifuge tube. Samples were stored in the freezer (-16^{o}C) to allow for pigment extraction for 24 hours and shaken once during this period. Samples were centrifuged for 20 minutes at 2000 rpm. 1 mL of solution was transferred using a glass pipette to a glass cuvette (1cm path length), and wavelengths were determined on a Pharmica LKB Ultrospec III Spectrophotometer. Wavelengths chosen for analysis were 647, 664, 610, 510, 480 nm. Chlorophyll analyses included determination of chlorophyll *a*, chlorophyll *b*, carotenoids, total chlorophyll (*a*+*b*) and ratio of chlorophyll *a*: *b*.

Water Quality Parameters – Water clarity was measured with a secchi disk. The secchi disk was lowered into the water until the black and white quarters could no longer be distinguished from one another. The depth at which this occurs is called the secchi depth. A TPS instrument was used to measure the pH, temperature and dissolved oxygen of the water and a portable refractometer was used to measure the salinity.

Water Column Herbicides – Water samples from cane drains in Bakers Creek and Fursden Creek were collected for analysis of herbicides in a 1L solvent washed glass container. Samples were also taken from the Pioneer River at 2 sites, one upstream and one at the mouth of the river, to determine if herbicides were present in the river water. Samples were analysed at QHSS. Methods for herbicide determination were the same as previously described for the mangrove core water pesticide determination.

Results

Assessment of Mangrove Condition and Dieback

Aerial Survey

Extent of Dieback in the Region

Mangrove areas were surveyed using a light aircraft in May 2002. This survey covered the six major estuarine systems from Reliance/Leila Creeks in the north to Sandringham Bay – Sandy/Alligator Creek in the south (Figures 2 and 3).

Extensive areas of moderate and severe dieback of *A. marina* were observed throughout the broader survey area. Dieback areas were closely comparable to those mapped in the preliminary survey in 2000 (Duke *et al.,* 2001), however the extent had changed. A thorough coverage with still photographs and video footage were used to increase validation accuracy and interpretation for the mapping of dieback condition in the aerial photographic survey.

The peculiar characteristics of the dieback condition were the same as that described in the preliminary survey. Notably again there was the unusual freckled appearance of affected mangrove canopies where dead individual trees of *A. marina* were scattered amongst normal living trees of mostly *R. stylosa* or *C. australis*. Dieback of *A. marina* extended from the water's edge up to the high water landward margin in all catchments surveyed, except Bucasia/Eimeo. In the latter, there was a minimal presence of *A. marina*, and no dead trees were observed.

Estimates of mangrove and dieback areas, based on 2002 survey results, are presented in Table 5, showing the area of mangroves, the proportion of the *A. marina* and non-*A. marina* and dieback condition. This data is also presented in Figure 7 as % mangrove area. Figure 6 shows the overall health classifications of *A. marina* for each estuary mapped.

Extent of Dieback in the Eimeo/Bucasia Creeks

There was no dieback of *A. marina* detected in Eimeo or Bucasia Creeks. The abundance of *A. marina* in these creeks was comparatively less than in the other estuaries mapped in this project. The areas of mangroves mapped in this area for this investigation were similar to the areas mapped in the CHRIS (Coastal Habitat Resources Information System database) (DPI, 2001) and the Winter and Wild (1995) report, but were significantly less than calculated in Digby *et al.,* (1999) (Table 4).

Extent of Dieback in the McCreadys Creek Estuary

Dieback of *A. marina* in McCreadys Creek was limited to a number of isolated patches (Figure 10C). Approximately 17% of *A. marina* trees were affected by moderate or severe dieback, which made up approximately 7% of total area of mangroves surveyed. The mangrove areas mapped were similar to those mapped by Winter and Wild (1995), Digby *et al.,* (1999) and the CHRIS database (DPI, 2001) (Table 4). Approximately 1% of species other than *A. marina* were affected by dieback, which made up approximately 0.1% of the total area of mangroves affected by dieback. This other type of dieback was comprised of mainly *C. australis.*

Extent of Dieback in the Pioneer River Estuary

Dieback occurred through all mangrove areas in the estuary. Dieback affected mangroves of all ages, as noted previously (Duke *et al.*, 2001), and stands from the river mouth to the upstream limits of mangroves in the Pioneer River (Figure 10F). The effect dominated most areas with few exceptions.

Curiously, the Sandfly Creek area was relatively free of severe dieback but this might reflect the greater change due to reclamation and alteration of the hydrology of this tributary. In this system there was relatively limited tidal exchange with river water through the modified drainage channel, limiting the presence of a possible water-borne agent responsible for the dieback. Another feature of these dieback areas is that they extend from creek margins to highwater landward margins. Generally, dieback was found where ever *A. marina* occurred, and there were few, if any, areas of healthy *A. marina* trees (Figure 9A-F).

Approximately 97% of *A. marina* trees in the Pioneer River estuary were affected by moderate or severe dieback, which equated to around 55% of total mangrove area. This also represents all areas occupied by *A. marina*, and the remaining areas appear unaffected only because they lack the species affected by the dieback agent.

Total mangrove areas were similar to those in the Oz Estuaries database (NLWRA, 2000) and the Winter and Wild (1995) estimates (Table 4). However, of other reports the areas calculated by Digby *et al.,* (1999) were much higher than those previously mentioned, and areas in the CHRIS database (DPI, 2001) areas were smaller.

Extent of Dieback in the Bakers Creek Estuary

Approximately 61% of *A. marina* trees in Bakers Creek were moderately or severely affected by the dieback and approximately 40% of the total area of mangroves were affected by the dieback. As with the McCreadys Creek mangroves, dieback in the Bakers Creek estuary was also patchy. There were two quite notable patches of severe dieback, both on the northern banks of the creek; one in the patch of mangroves at the mouth of the estuary (Figure 10B), and one very large patch of mangroves around the first bend of the creek. Areas of mangroves calculated in this assessment were similar to Oz Estuaries Database (NLWRA, 2000) and Winter and Wild (1995) but was significantly smaller than Digby *et al.,* (1999) and greater than areas calculated with the CHRIS database (DPI, 2001) (Table 4). There was less than 1 hectare of dieback of species other than *A. marina* in Bakers Creek.

TABLE 4: Comparison of mangrove areas mapped (hectares) in Bucasia Creek, Eimeo Creek, McCreadys Creek, Pioneer River and Bakers Creek in five different mapping exercises in the Mackay Region. Blank entries indicate no mapping exercise was done in that area. * (Bassett Basin only)

	Current Investigation (2002)	**NLWRA (2000)**	**Winter and Wild 1995 (1993)**	**Digby *et al.,* 1999**	**DPI (2001)**
Bucasia Creek	165		585	300	146
Eimeo Creek	30				27
McCreadys Creek	415			430	391
Pioneer River	624	645	619 *	760	541
Bakers Creek	436	410	447	730	345

TABLE 5: Areas of mangroves classified as no dieback (<15% dead and sick *A. marina*), moderate (15-30%) and severe (>30%), in the Mackay Region, including Bucasia and Eimeo Creeks, McCreadys Creek, Pioneer River Estuary and Bakers Creek.

	Mangrove areas (ha) for 3 levels of health			
Bucasia Creek	**None**	**Moderate**	**Severe**	**Total**
A. marina	15	0	0	**15**
Non-*A. marina*	150	0	0	**150**
Total Mangrove				**165**
Eimeo Creek	**None**	**Moderate**	**Severe**	**Total**
A. marina	10	0	0	**10**
Non-*A. marina*	20	0	0	**20**
Total Mangrove				**30**
McCreadys Creek	**None**	**Moderate**	**Severe**	**Total**
A. marina	154	16	15	**185**
Non-*A. marina*	227	3	0	**230**
Total Mangrove				**415**
Pioneer River	**None**	**Moderate**	**Severe**	**Total**
A. marina	11	58	288	**357**
Non-*A. marina*	267	0	0	**267**
Total Mangrove				**624**
Bakers Creek	**None**	**Moderate**	**Severe**	**Total**
A. marina	109	75	98	**282**
Non-*A. marina*	154	0	0	**154**
Total Mangrove				**436**

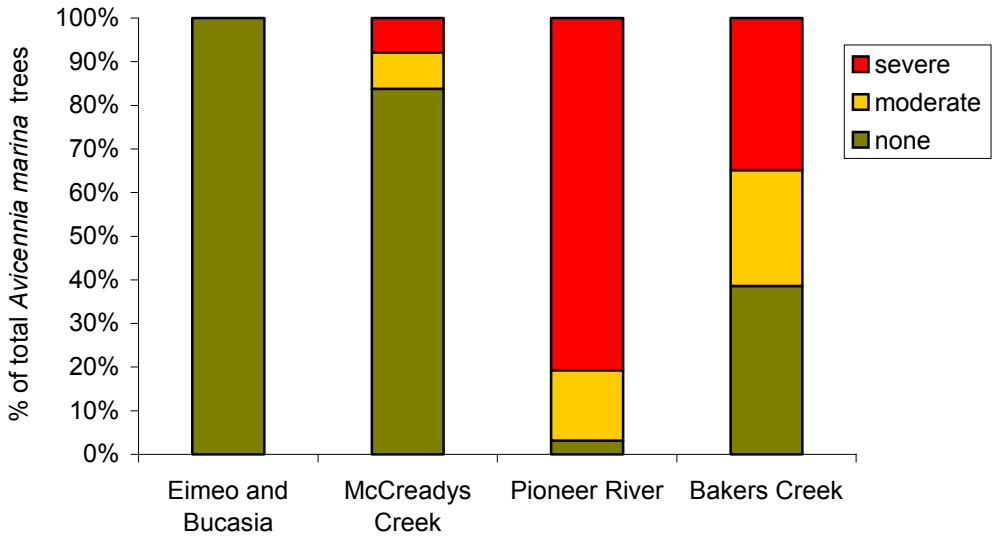

FIGURE 7: Health of *A. marina* in Eimeo and Bucasia, McCreadys Creek, Pioneer River and Bakers Creek as quantified by the mapping exercise in 2002.

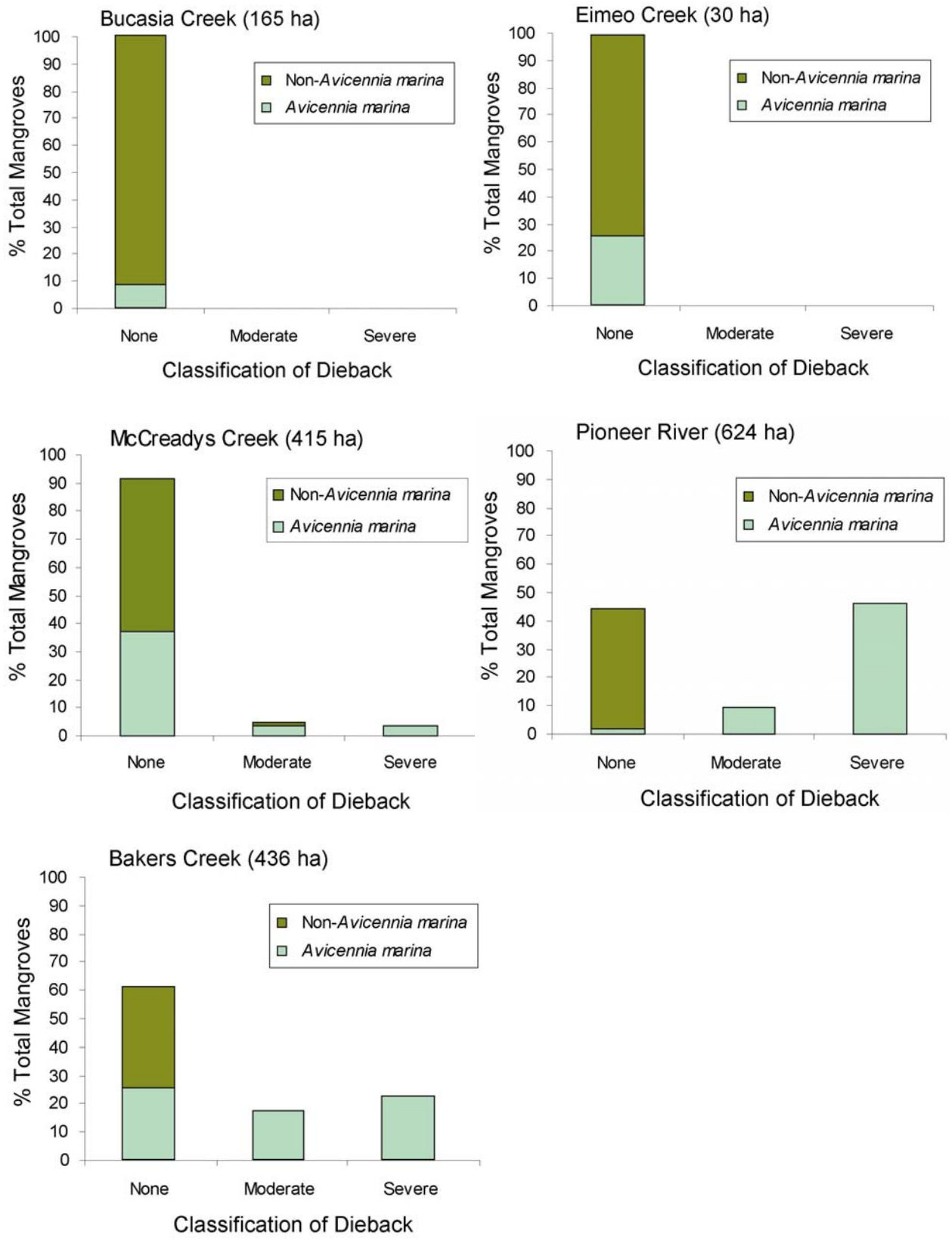

FIGURE 8: Classification of mangroves from Bucasia Creek, Eimeo Creek, McCreadys Creek, Pioneer River and Bakers Creek using aerial photographs, into areas of *A. marina* (mixed communities) and non-*A. marina* trees and their dieback classification (none, moderate or severe).

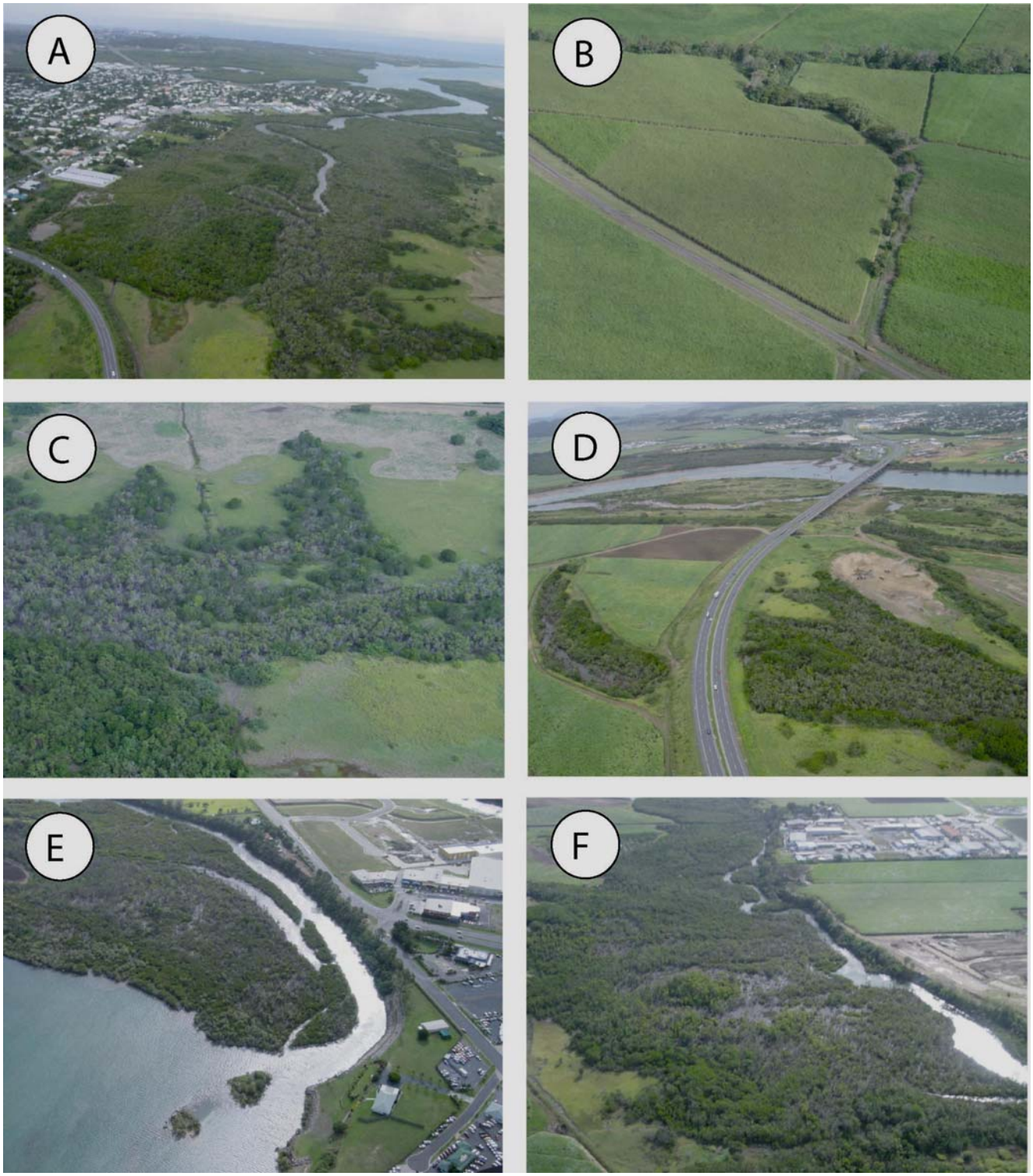

FIGURE 9: (A) Severe dieback in Barnes Creek with Mackay city in the background (B) A cane drain in the Pioneer River estuary that drains into Fursden Creek (C) Mangrove dieback at the source of Barnes Creek (D) View across the Pioneer River to Fursden Creek, with isolated patch of mangroves in on the other side of the road with dieback (E) Mouth of Fursden Creek, Pioneer River (F) Severe degradation of mangrove habitat within Fursden Creek.

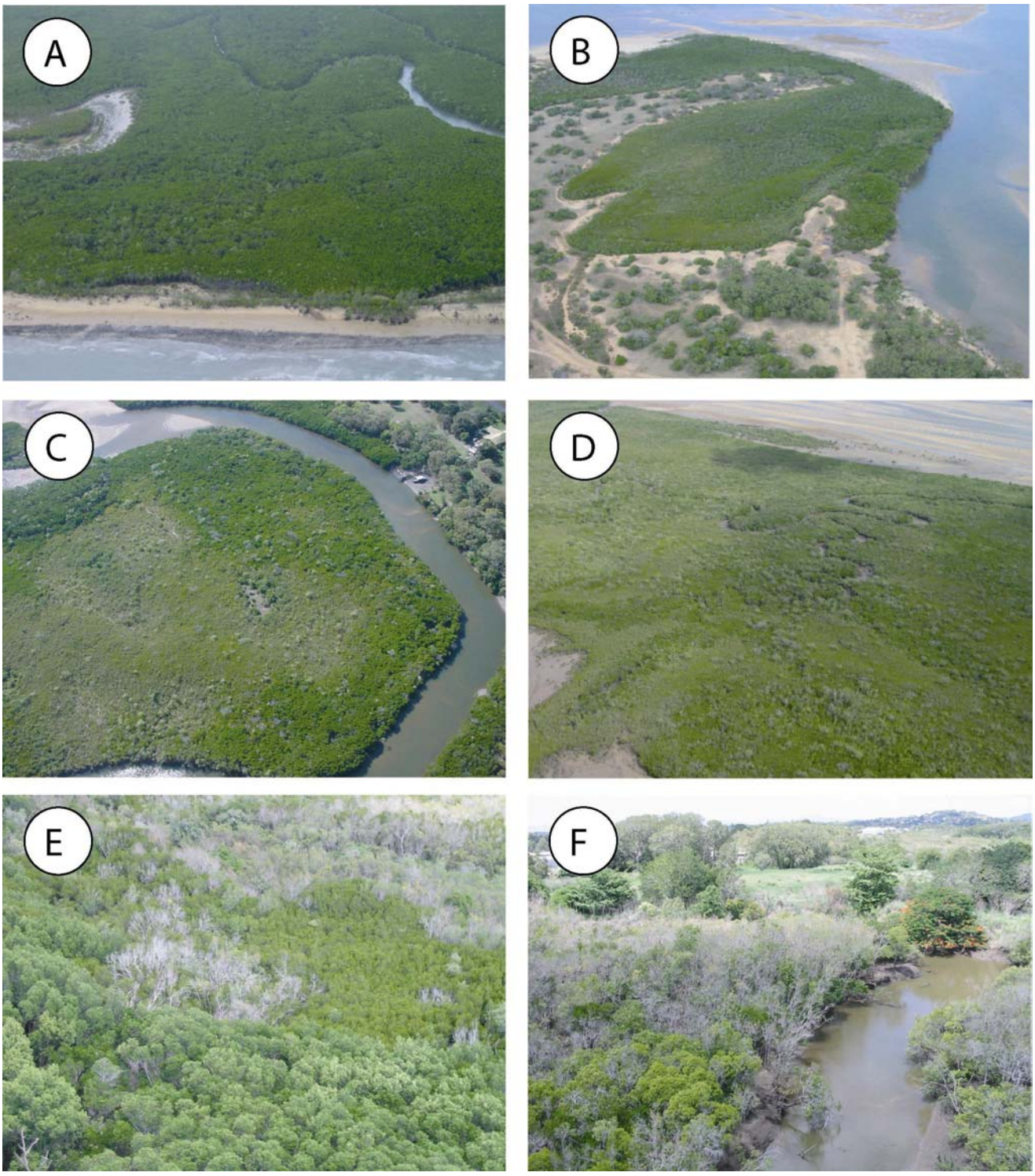

FIGURE 10: (A) Reliance Creek to the north of Bucasia, with patches of *A. marina* dieback amongst *C. australis* (B) Bakers Creek with severe *A. marina* dieback at the mouth (C) McCreadys Creek with moderate freckled dieback (D) Sandringham Bay with freckled dieback (E) Severe dieback of *A. marina* mixed among live stands of other species, in the Pioneer River (Photo J. Wake and K. Dodd) (F) *A. marina* dieback in an upstream location in the Pioneer River (Photo J. Wake and K. Dodd).

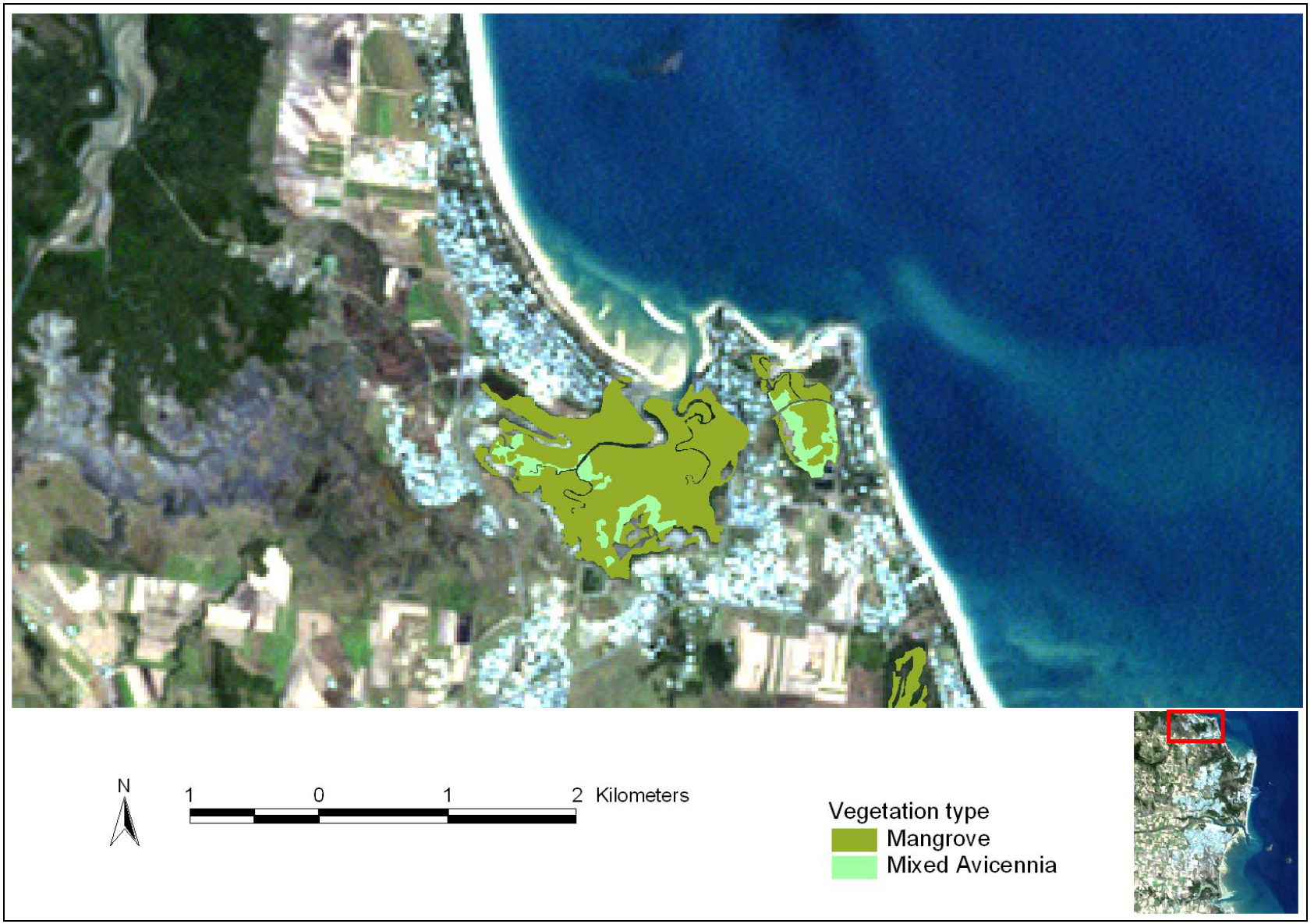

FIGURE 11: Vegetation map of Eimeo and Bucasia Creeks displaying mangrove (without *A. marina*) distribution [green] and *A. marina* distribution [orange] (mixed communities).

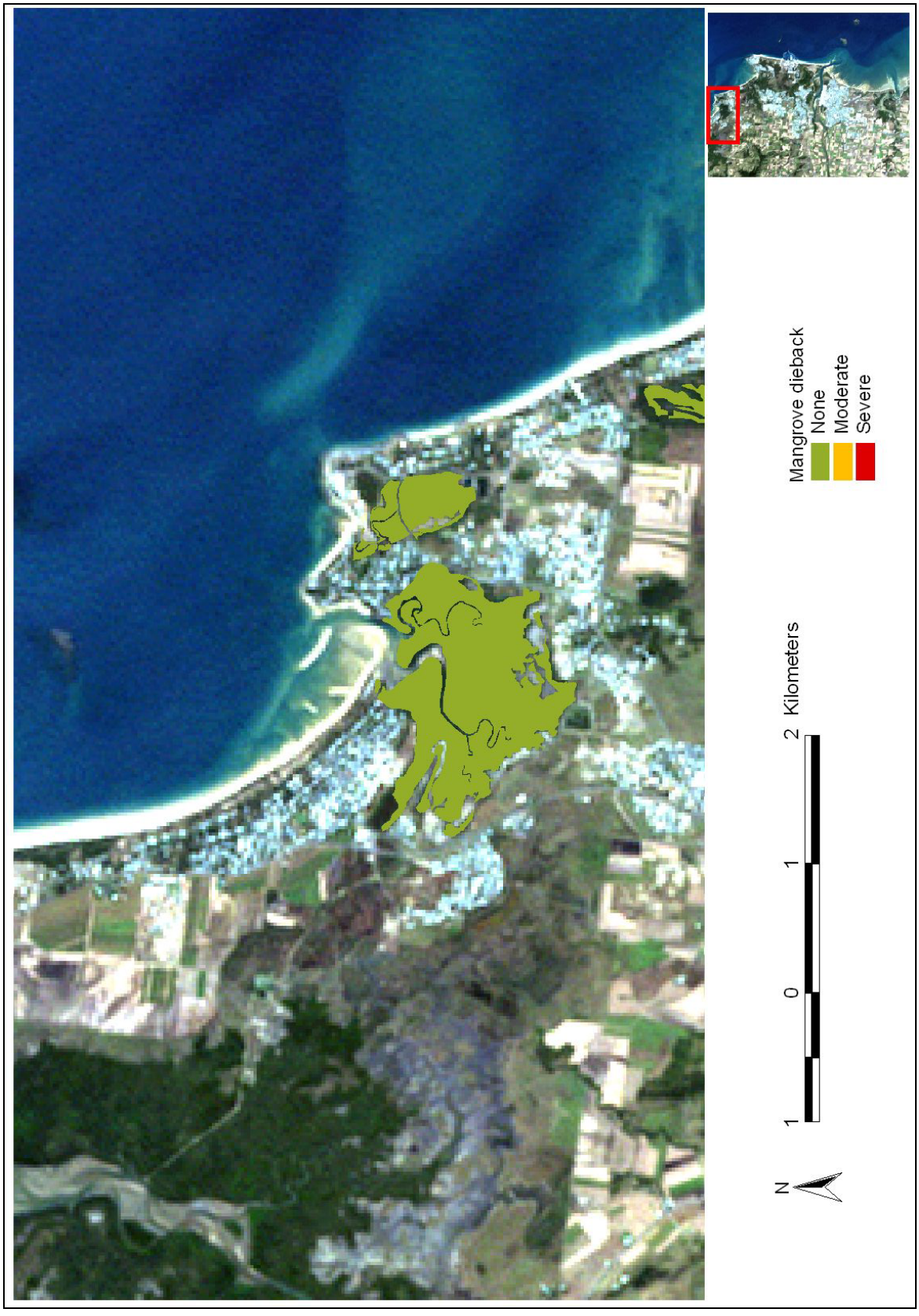

FIGURE 12: Classified vegetation map of Eimeo and Bucasia Creeks, showing mangrove health categories: None (no *A. marina* dieback), Moderate (less than 30% dieback) and Severe (greater than 30% dieback).

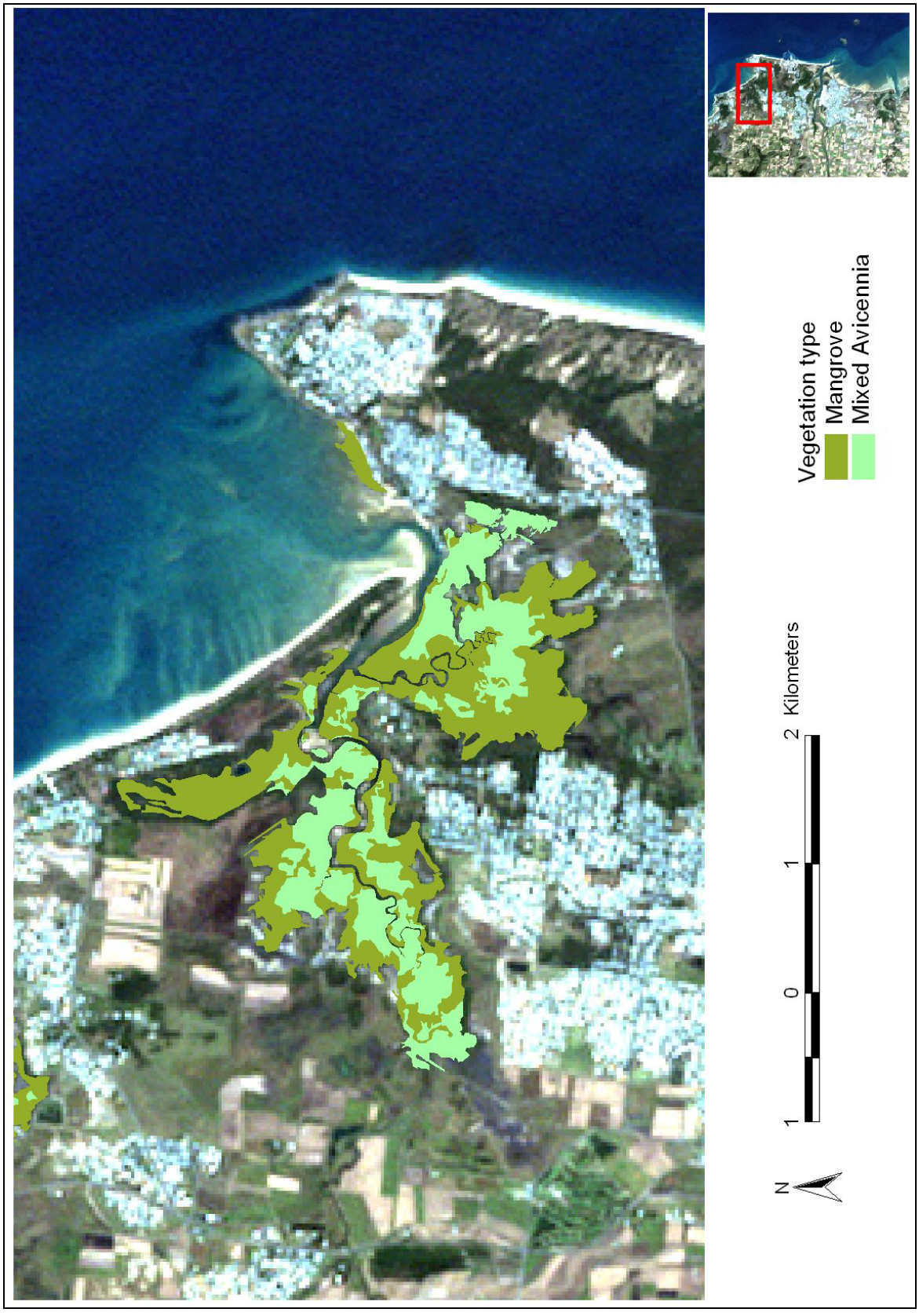

FIGURE 13: Vegetation map of McCreadys Creek displaying mangrove (without *A. marina*) distribution [green] and *A. marina* distribution [orange] (mixed communities).

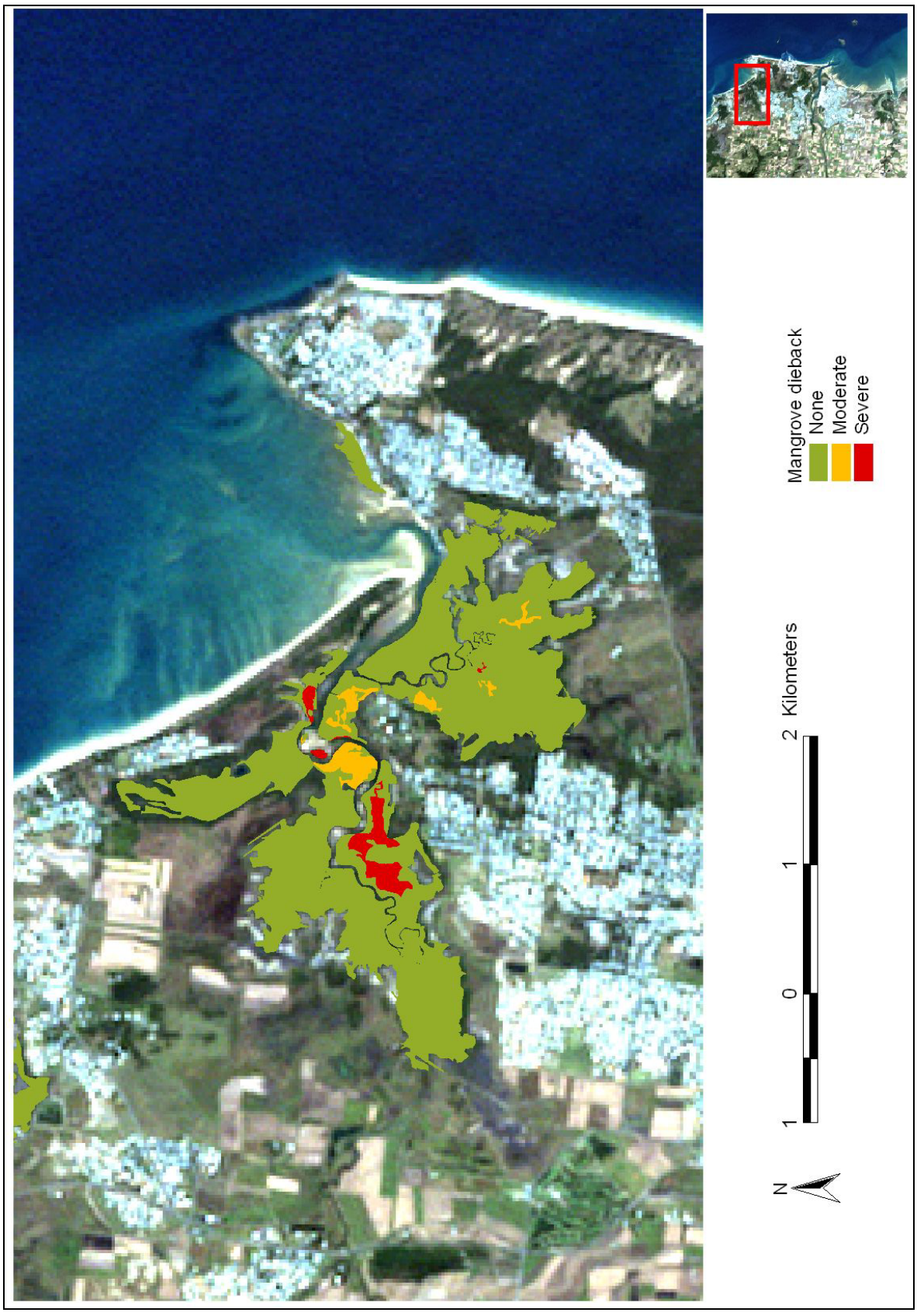

FIGURE 14: Classified vegetation map of McCreadys Creek, showing mangrove health categories: None (no *A. marina* dieback), Moderate (less than 30% dieback) and Severe (greater than 30% dieback).

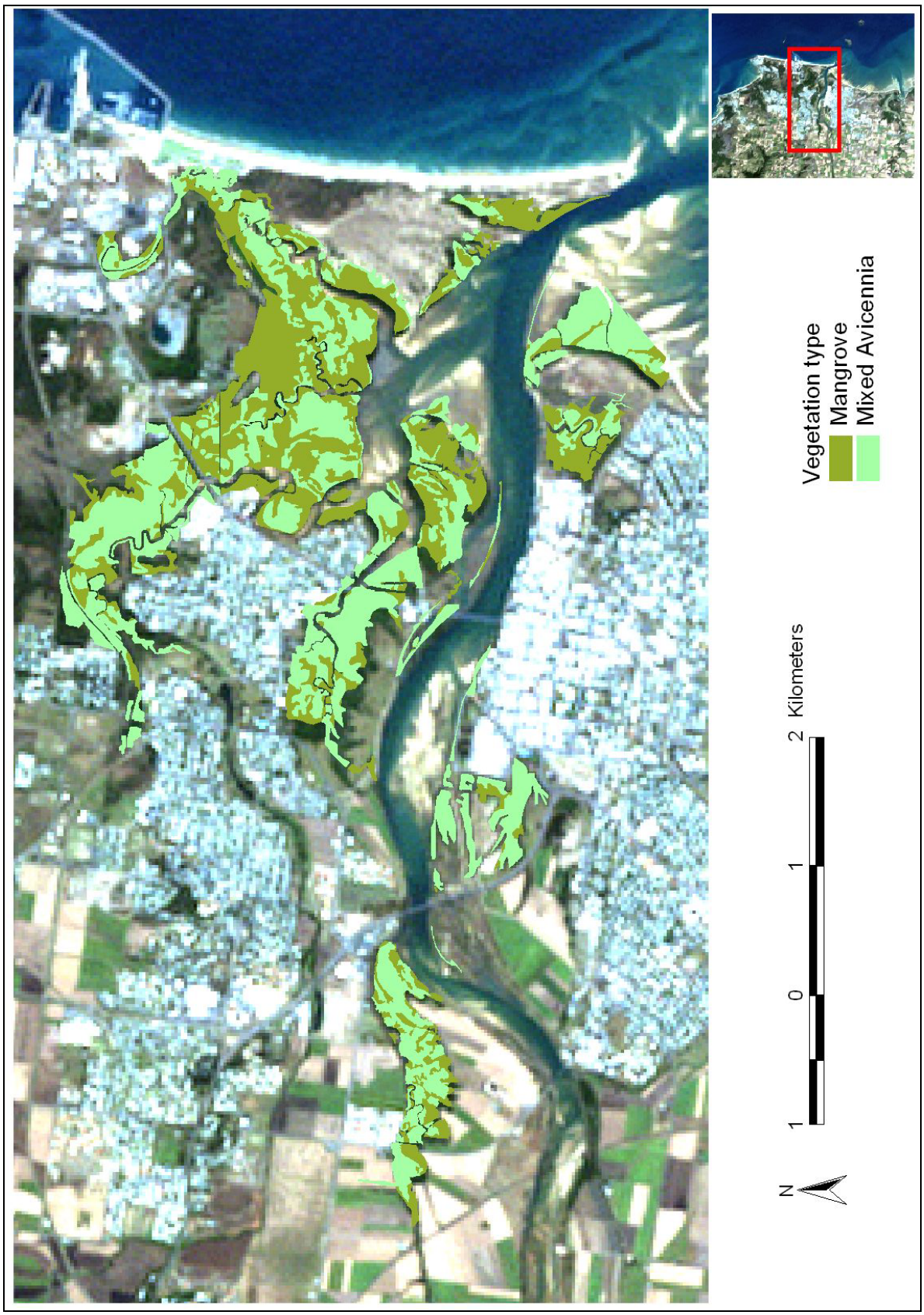

FIGURE 15: Vegetation map of the Pioneer River estuary displaying mangrove (without *A. marina*) distribution [green] and *A. marina* distribution [orange] (mixed communities).

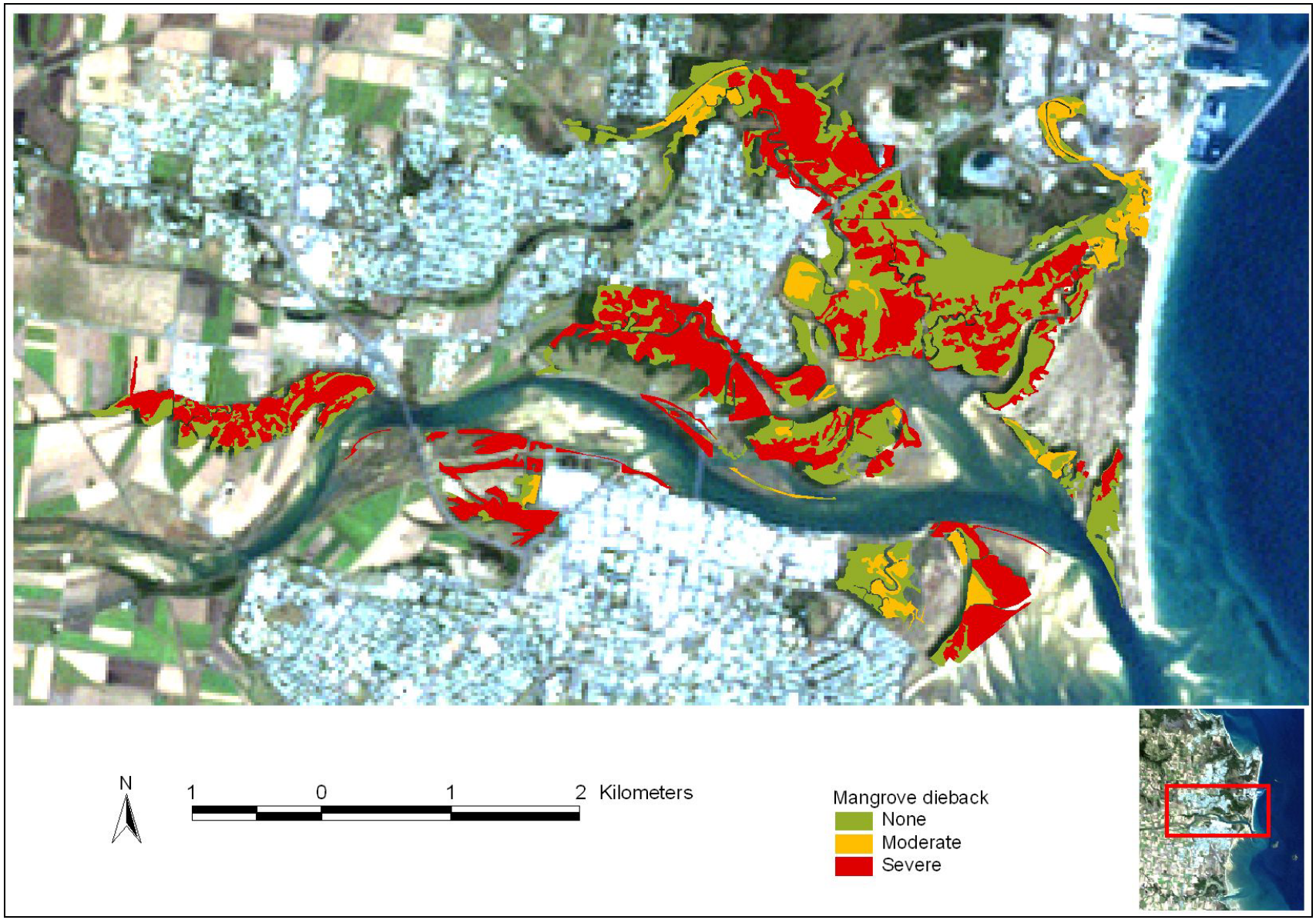

FIGURE 16: Classified vegetation map of the Pioneer River estuary, showing mangrove health categories: None (no *A. marina* dieback), Moderate (less than 30% dieback) and Severe (greater than 30% dieback).

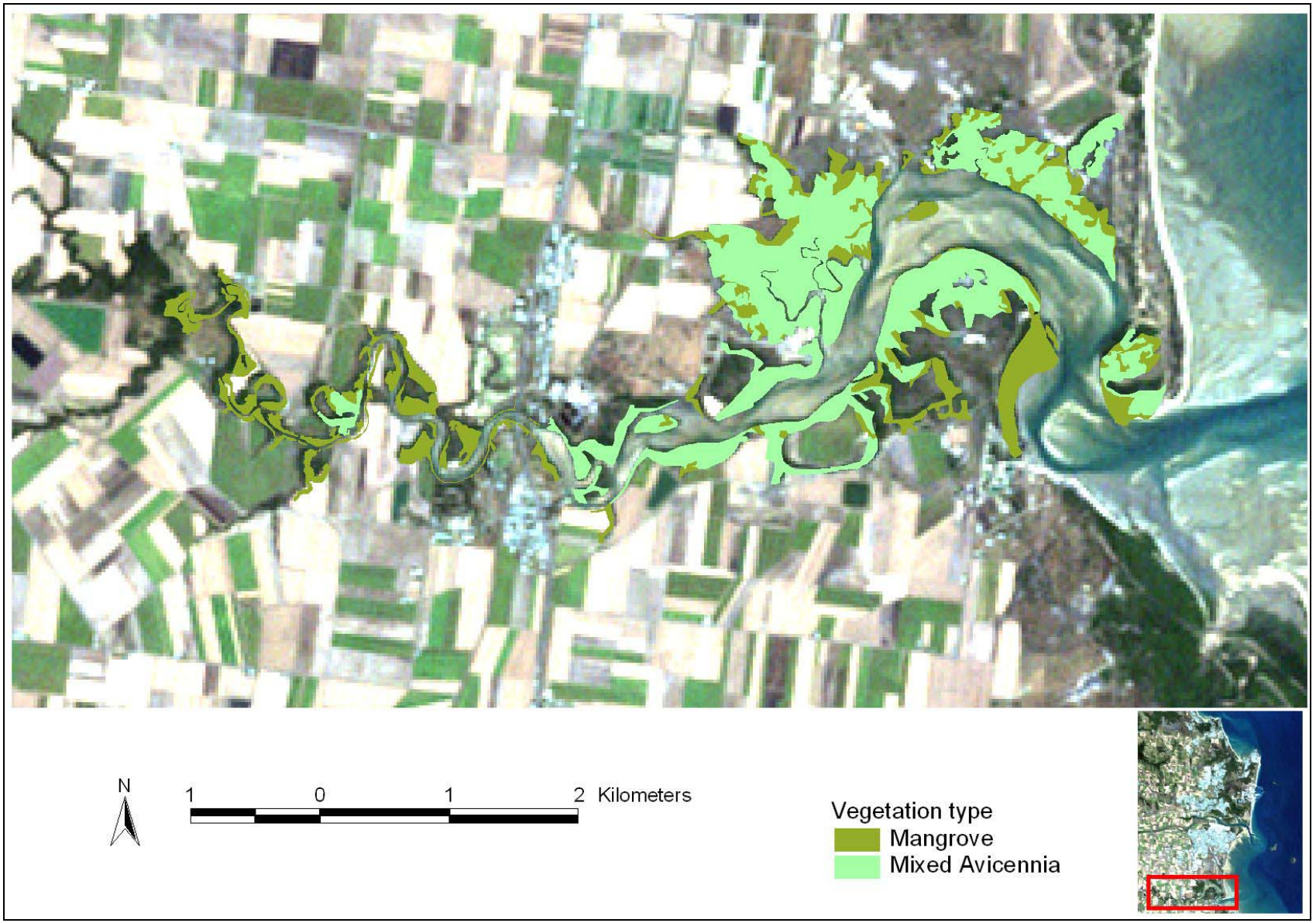

FIGURE 17: Vegetation map of the Bakers Creek estuary displaying mangrove (without *A. marina*) distribution [green] and *A. marina* distribution [orange] (mixed communities).

FIGURE 18: Classified vegetation map of Bakers Creek, showing mangrove health categories: None (no *A. marina* dieback), Moderate (less than 30% dieback) and Severe (greater than 30% dieback).

Ground Survey of Mangrove Condition

Mangrove Plots – Tree Condition

Since the dieback condition under investigation chiefly affected *A. marina* (Duke at al., 2001), all plots were chosen to include *A. marina* trees. The dominance of *A. marina* in these plots however varied depending on both the normal ecophysiological conditions and the presence of the dieback agent. The relative compositions of each of these plots are shown in Figure 19. The majority of sites within Barnes Creek (with the exception of BS1 and BM1) were dominated by living *A. marina* trees. Plots at McCreadys Creek were more mixed. Several plots in Bakers Creek, McCreadys Creek and Eimeo had greater numbers of other species.

Tree condition and the locations of plots in each of the three estuaries, McCreadys Creek, Barnes Creek in Pioneer River, Bakers Creek, are presented in Table 6 and Figures 20 to 22.

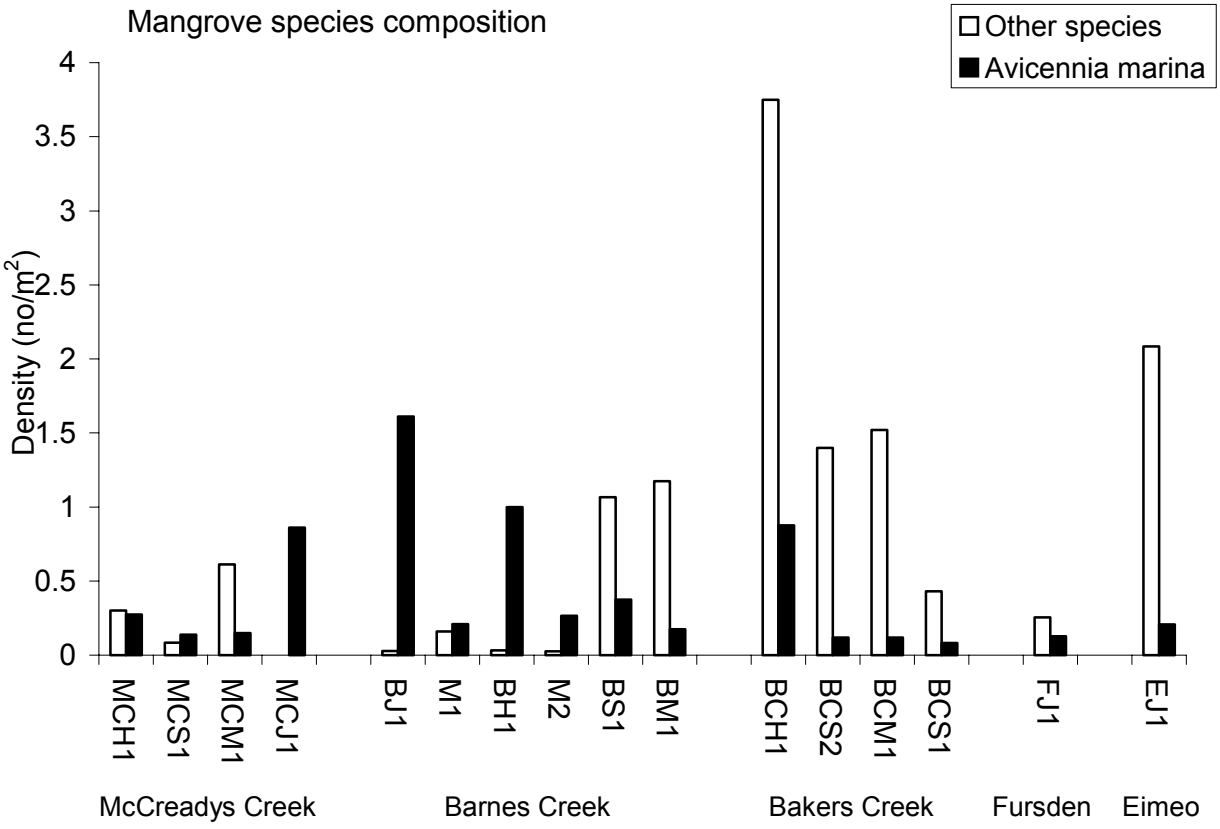

FIGURE 19: Density of living mangroves (number/m^2) in McCreadys Creek, Barnes Creek, Bakers Creek, Fursden Creek and Eimeo Creek comparing the density of *A. marina* to other species in each of the plots. Refer to Figures 4, 5 and 6 for site locations.

TABLE 6: Percentage composition of *A. marina* as healthy, unhealthy (sick) or dead in McCreadys Creek, Barnes Creek, Bakers Creek, Fursden Creek and Eimeo. Overall stand health of *A. marina* was classified using standards set by Duke *et al*., (2001). Refer to Figures 4,5 and 6 for site locations.

Demography and Condition of Trees		Site Code	# Trees in Plot	Tree Density (#/m^2)	% *A. marina* in plot	Condition of *A. marina* trees (% AM)			Condition of Non-*A. marina* (%)			Dieback Classification
No.	**Site**					**Healthy**	**Sick**	**Dead**	**Healthy**	**Sick**	**Dead**	
1	**McCreadys Creek**	**MCJ1**	**27**	0.8	100	85	6	9	-	-	-	None
2	**McCreadys Creek**	**MCH1**	**26**	0.7	46	83	8	8	86	0	14	Moderate
3	**McCreadys Creek**	**MCM1**	**64**	0.8	23	53	27	20	100	0	0	Severe
4	**McCreadys Creek**	**MCS1**	**25**	0.7	36	56	0	44	0	19	81	Severe
5	**Barnes Creek**	**BH1**	**35**	1.2	97	35	53	12	100	0	0	Severe
6	**Barnes Creek**	**BM1**	**58**	1.5	19	18	45	36	40	57	2	Severe
7	**Barnes Creek**	**M1**	**44**	0.4	84	38	19	43	83	17	0	Severe
8	**Barnes Creek**	**M2**	**32**	0.4	94	30	37	33	100	0	0	Severe
9	**Barnes Creek**	**BS1**	**65**	1.7	31	20	50	30	16	76	9	Severe
10	**Barnes Creek**	**BJ1**	**68**	1.9	99	78	9	13	100	0	0	Moderate
11	**Bakers Creek**	**BCH1**	**78**	4.9	23	78	0	22	98	2	0	Moderate
12	**Bakers Creek**	**BCM1**	**52**	2.1	23	17	8	75	69	31	0	Severe
13	**Bakers Creek**	**BCS1**	**178**	3.7	6	10	30	60	4	7	89	Severe
14	**Bakers Creek**	**BCS2**	**52**	2.1	19	30	0	70	79	5	17	Severe
15	**Fursden Creek**	**FJ1**	**30**	0.6	57	18	12	71	83	0	17	Severe
16	**Eimeo**	**EJ1**	**59**	2.5	8	100	0	0	93	0	7	None

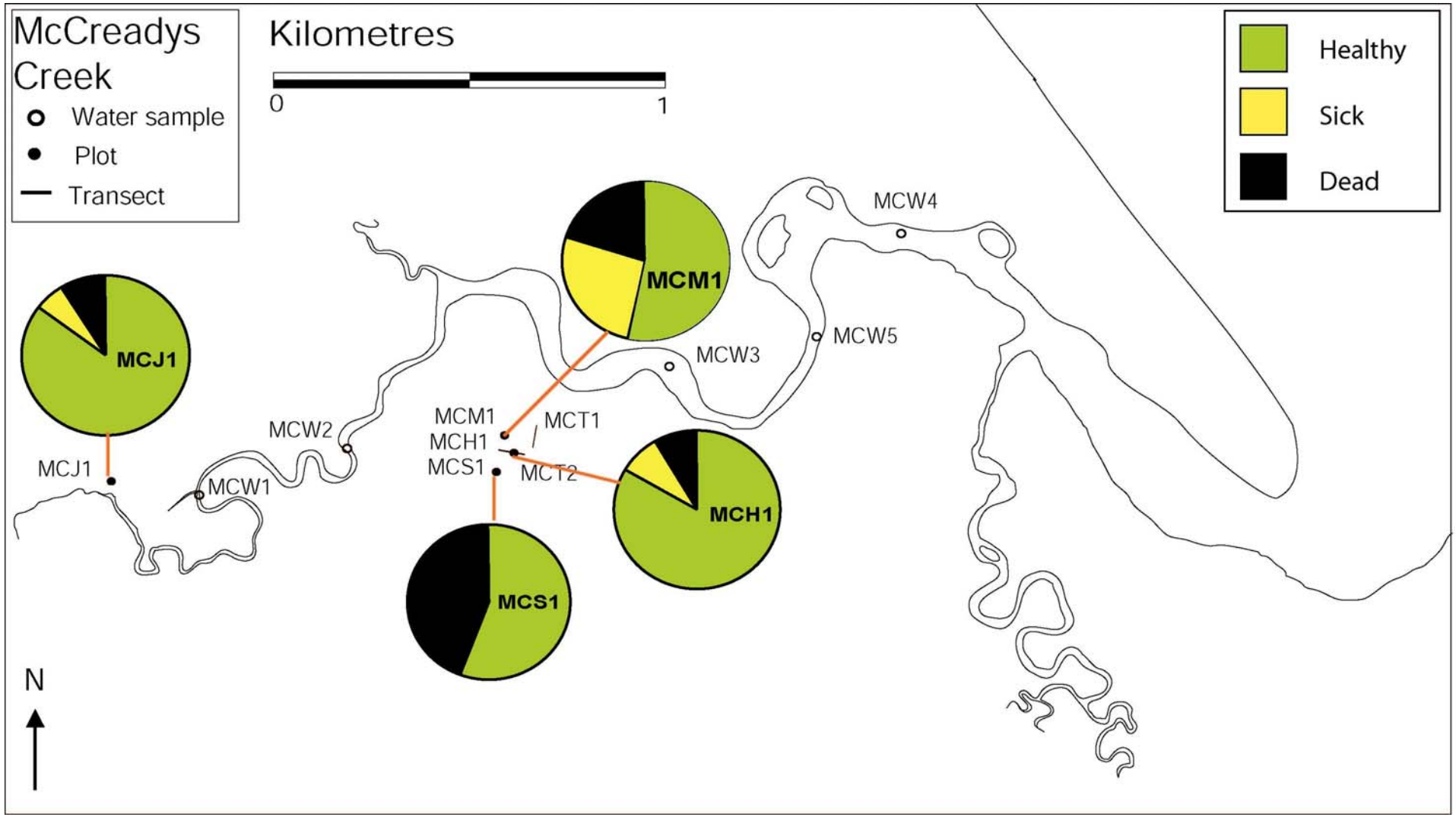

FIGURE 20: Proportion of *A. marina* trees as healthy, unhealthy (sick) and dead from mangrove plots in McCreadys Creek.

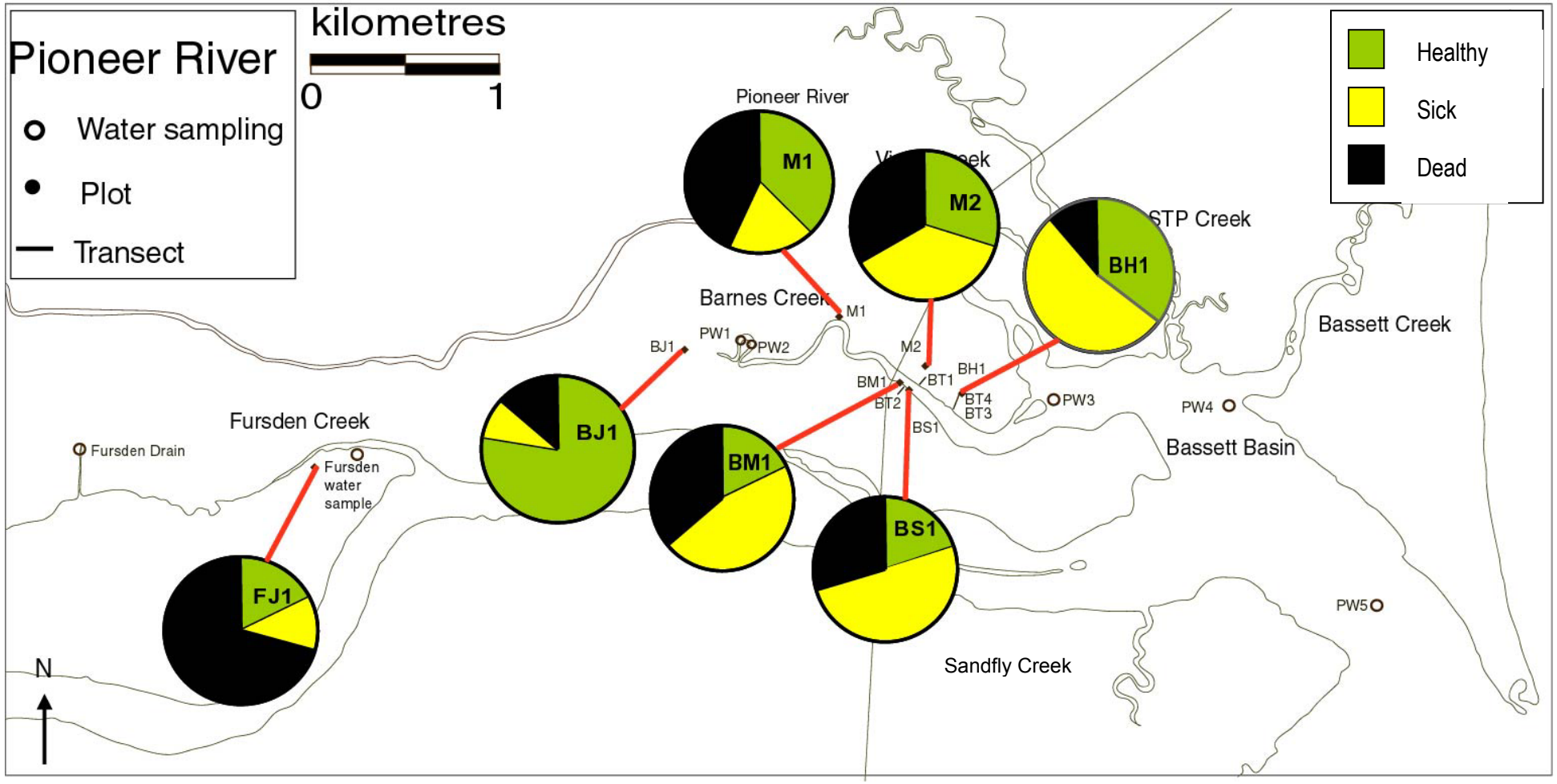

FIGURE 21: Proportion of *A. marina* trees as healthy, unhealthy (sick) and dead from mangrove plots in the Pioneer River estuary.

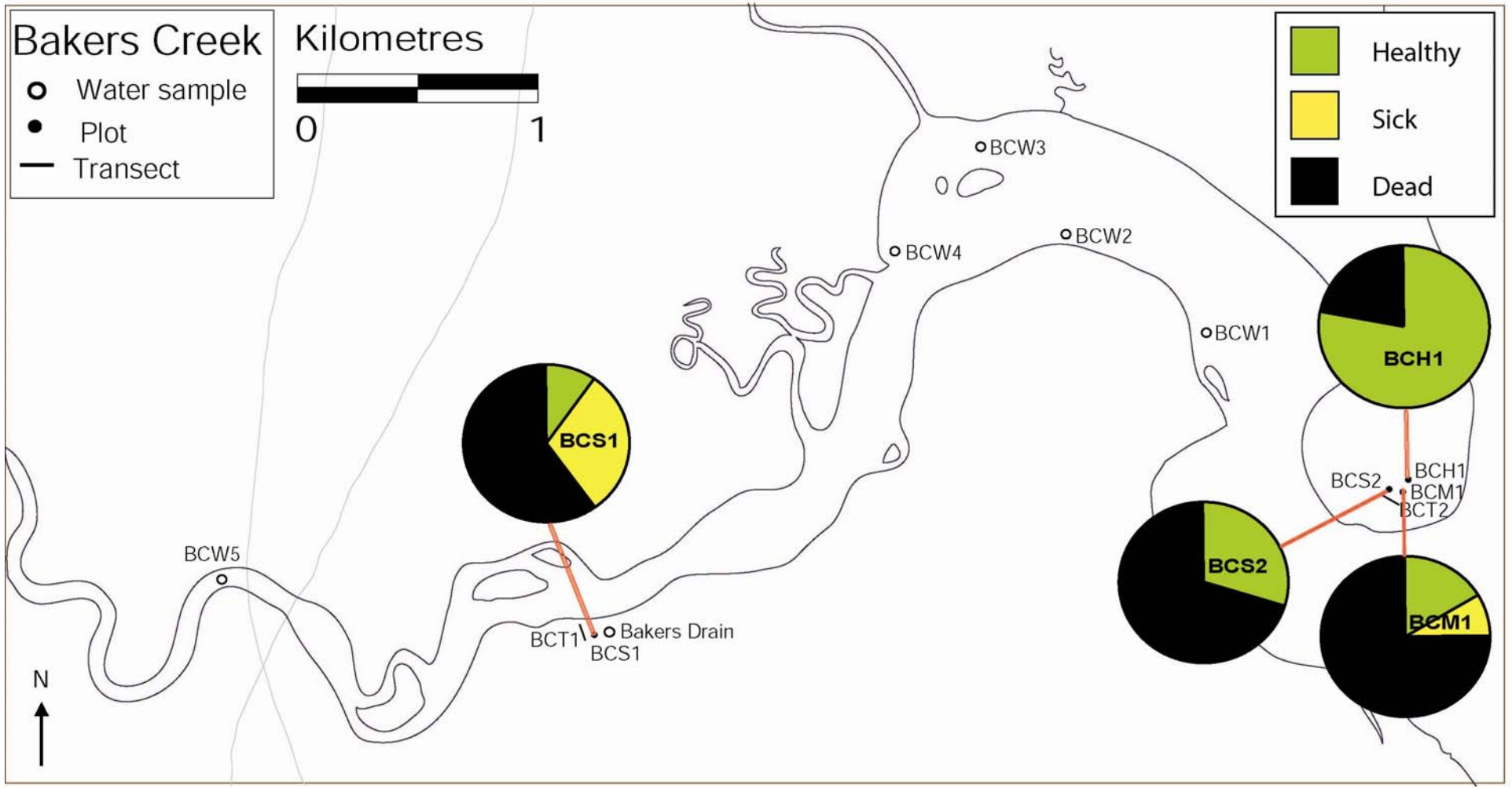

FIGURE 22: Proportion of *A. marina* trees as healthy, unhealthy (sick) and dead from mangrove plots in Bakers Creek.

McCreadys Creek

A. marina trees were proportionally healthier in plots in McCreadys Creek than in Barnes Creek and Bakers Creek (Figure 20). This was confirmed by the aerial mapping study (Table 3). There were also patches of localised dieback of mangrove species other than *A. marina* in McCreadys Creek. This dieback was represented in the plot MCS1, which had a large number of dead and sick *C. australis*, but no unhealthy (sick) *A. marina* trees (Figure 23). This *C. australis* dieback was observed in the aerial photography.

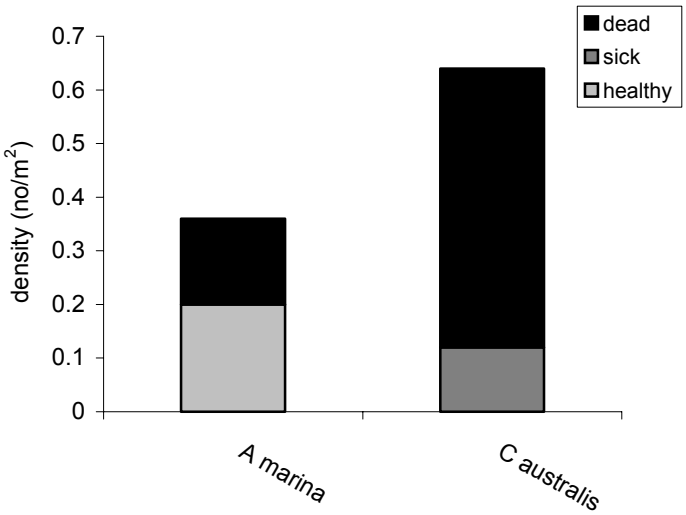

FIGURE 23: Mangrove plot MCS1 in McCreadys Creek showing proportions of healthy, unhealthy (sick), and dead trees of *A. marina* and *C. australis* in each plot. Refer to Figures 4,5 and 6 for site locations.

Pioneer River

In the Pioneer River, all plots excluding plot BJ1 had dieback that was classified as severe. The severity of dieback was confirmed in the aerial mapping exercise (Figures 15 and 16). There were a large proportion of sick trees within the majority of plots, which suggests that the agent causing the dieback was still present. Although *A. marina* was the only species to suffering from widespread dieback, there were instances where other mangrove species were observed to be unhealthy, noting in particular the mangrove plots BS1 and BM1. In plot BS1, both the *A. corniculatum* and *A. marina* were unhealthy, with yellow leaves and dead branches, while there were healthy *R. stylosa* trees present in the plot. Plot BM1 was located in close vicinity to BS1. Again, *A. marina* and *A. corniculatum* appeared to be unhealthy, with yellowing leaves. Some *C. australis*, *Exoecaria agallocha* and *R. stylosa* also had yellowing leaves. The sickness observed in other species was not detected in the aerial mapping exercise.

Bakers Creek

Within Bakers Creek there was one moderate dieback plot, while the rest were classified as severe. Plots within Bakers Creek had large proportions of dead trees, but not as many sick trees, particularly near the mouth of the creek. There were two plots in Bakers Creek with dieback of *C. australis*. These were BCS1 (Figure 24), with large-scale dieback of *C. australis* and BCS2 with a comparatively smaller amount of *C. australis* dieback. This dieback of *C. australis* was not widespread throughout the entire Bakers Creek estuary however.

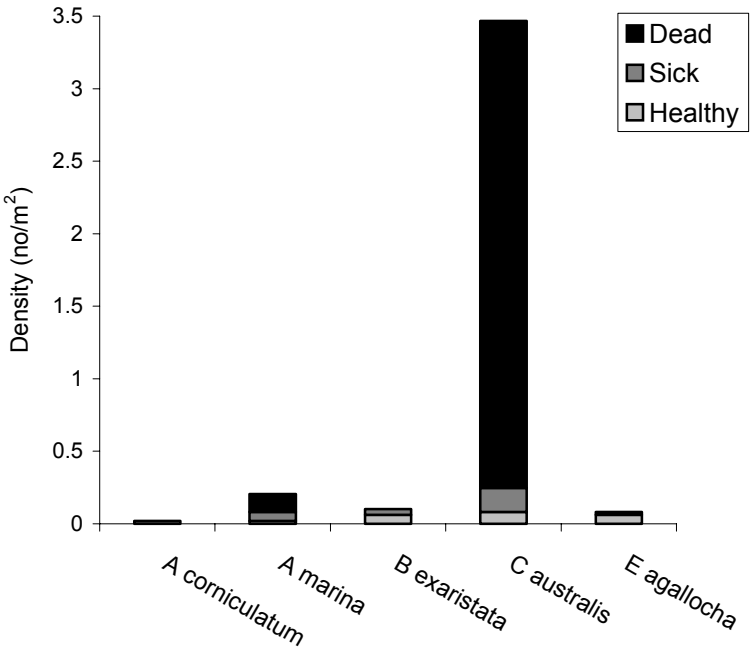

FIGURE 24: Mangrove plot BCS1 in Bakers Creek showing proportions of healthy, unhealthy (sick), and dead trees in each plot. Refer to Figures 4,5 and 6 for site locations.

Mangrove Plots – Seedling Condition

The condition, density and composition of mangrove seedlings in each plot are presented in Table 7. Seedlings of species other than *A. marina* were generally healthy in each of the plots except for the seedlings in the plot BS1 in Barnes Creek (Figure 25), which were unhealthy (sick) with yellowing leaves in all species (*A. corniculatum* and *R. stylosa*). This was the same plot in which adult trees of *A. corniculatum* were affected.

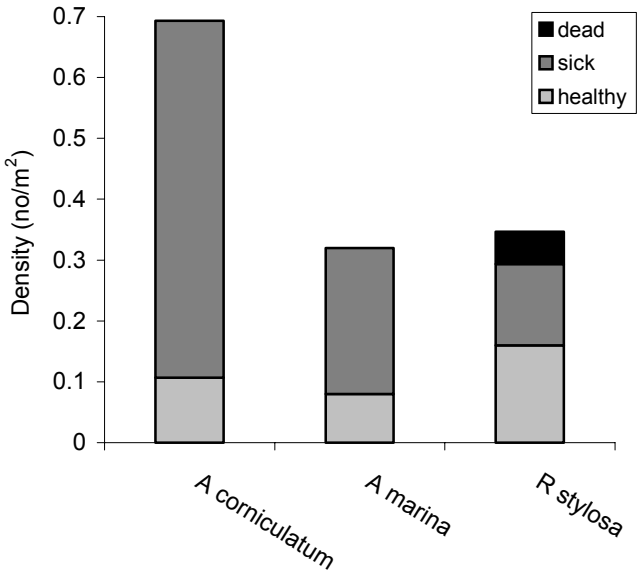

FIGURE 25: Density of seedlings in *A. corniculatum*, *A. marina* and *R. stylosa*, showing their respective health classification at site BS1 in Barnes Creek, Pioneer River estuary. Refer to Figures 4, 5 and 6 for site locations.

TABLE 7: Seedling density, composition and condition in mangrove plots in McCreadys Creek, Pioneer River (Barnes Creek and Fursden Creek), Bakers Creek and Eimeo. *A. marina* were identified as healthy, unhealthy (sick) or dead in each plot. Refer to Figures 4, 5 and 6 for site locations.

	Demography and Condition of Seedlings	Site Code	# Seedlings in Plot	Density (#/m2)	Seedling Composition (% *A. marina*)	*A. marina* seedlings: trees (Ratio)	Condition of *A. marina* (% AM seedlings)		
	Site						**Healthy**	**Sick**	**Dead**
1	**McCreadys Creek**	**MCJ1**	75	2.1	33	0.9	44	56	0
2	**McCreadys Creek**	**MCH1**	40	1.0	95	3.2	97	3	0
3	**McCreadys Creek**	**MCM1**	33	1.7	21	0.5	43	57	0
4	**McCreadys Creek**	**MCS1**	13	0.4	92	1.3	67	33	0
5	**Barnes Creek**	**BH1**	9	1.2	100	0.3	67	33	0
6	**Barnes Creek**	**BM1**	24	0.6	71	2.2	76	24	0
7	**Barnes Creek**	**M1**	24	0.2	75	0.6	94	6	0
8	**Barnes Creek**	**M2**	13	0.2	54	0.4	100	0	0
9	**Barnes Creek**	**BS1**	51	1.4	24	2.6	75	25	0
10	**Barnes Creek**	**BJ1**	47	1.3	100	0.7	85	6	9
11	**Bakers Creek**	**BCH1**	15	0.9	7	0.1	100	0	0
12	**Bakers Creek**	**BCM1**	45	1.8	56	2.1	72	28	0
13	**Bakers Creek**	**BCS1**	15	0.3	33	0.5	0	100	0
14	**Bakers Creek**	**BCS2**	52	2.1	0	0.0	-	-	-
15	**Fursden Creek**	**FJ1**	14	0.3	64	0.5	78	11	11
16	**Eimeo Creek**	**EJ1**	173	7.2	27	9.4	71	8	21

Transect Data

The transect data shows that there was severe dieback in all of the estuaries surveyed, with the majority of *A. marina* along each transect being classified as severe. There were a larger proportion of healthy *A. marina* trees inland. Above-ground pneumatophore height data is presented alongside the transect data where available, showing that dieback was not correlated with pneumatophore height.

McCreadys Creek Transect 1 (MCT1)

A. marina increased in health from mid-mangroves to the terrestrial margin of the mangrove forests (Figure 26a). This transect was dominated by *A. marina*, *R. stylosa* and *C. australis*. There was no relationship between pneumatophore height and health of *A. marina* along the transect (Figure 26b).

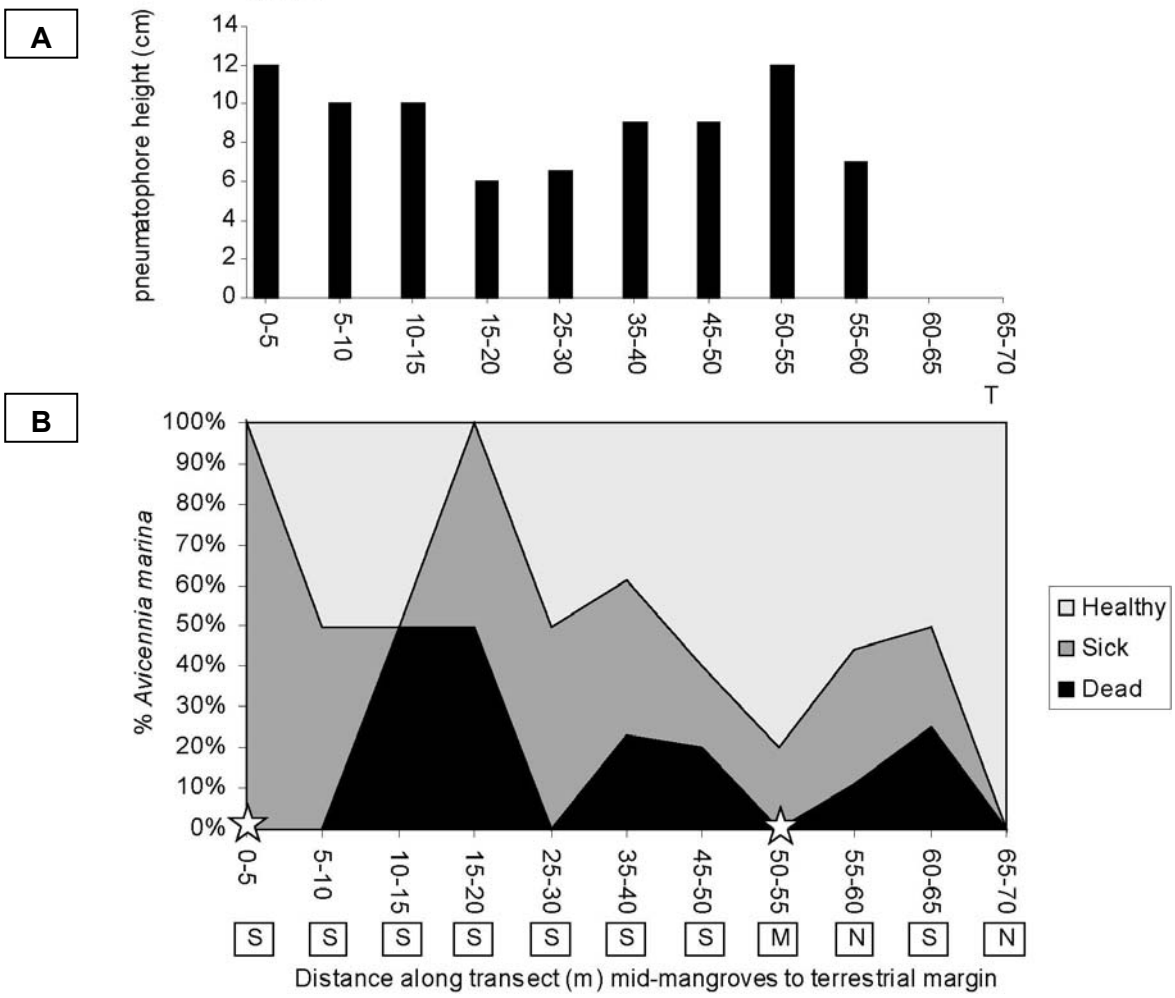

FIGURE 26: McCreadys Creek transect 1 (MCT1). **(A)** Above-ground pneumatophore heights along the transect **(B) C**ondition of mangroves (healthy, sick or dead) and proportion of *A. marina* were represented in plots along the transect, which was run from the creek to the terrestrial margin (T) of the mangroves.. *A. marina* mangroves were classified as having no dieback (N), moderate (M) or severe (S) dieback based upon their condition in the plot. A sample for herbicide analysis was taken from the core water (star). Results for herbicides in this transect are displayed in Table 15. For information on the location of the transect, see Figure 4.

McCreadys Creek Transect 2 (MCT2)

A. marina health increased in health from the creek edge to the more landward areas of mangroves (Figure 27b). This transect was dominated by *A. marina*, *R. stylosa* and *C. australis.* The pneumatophore height data did not show any trends with the health of *A. marina* (Figure 27A).

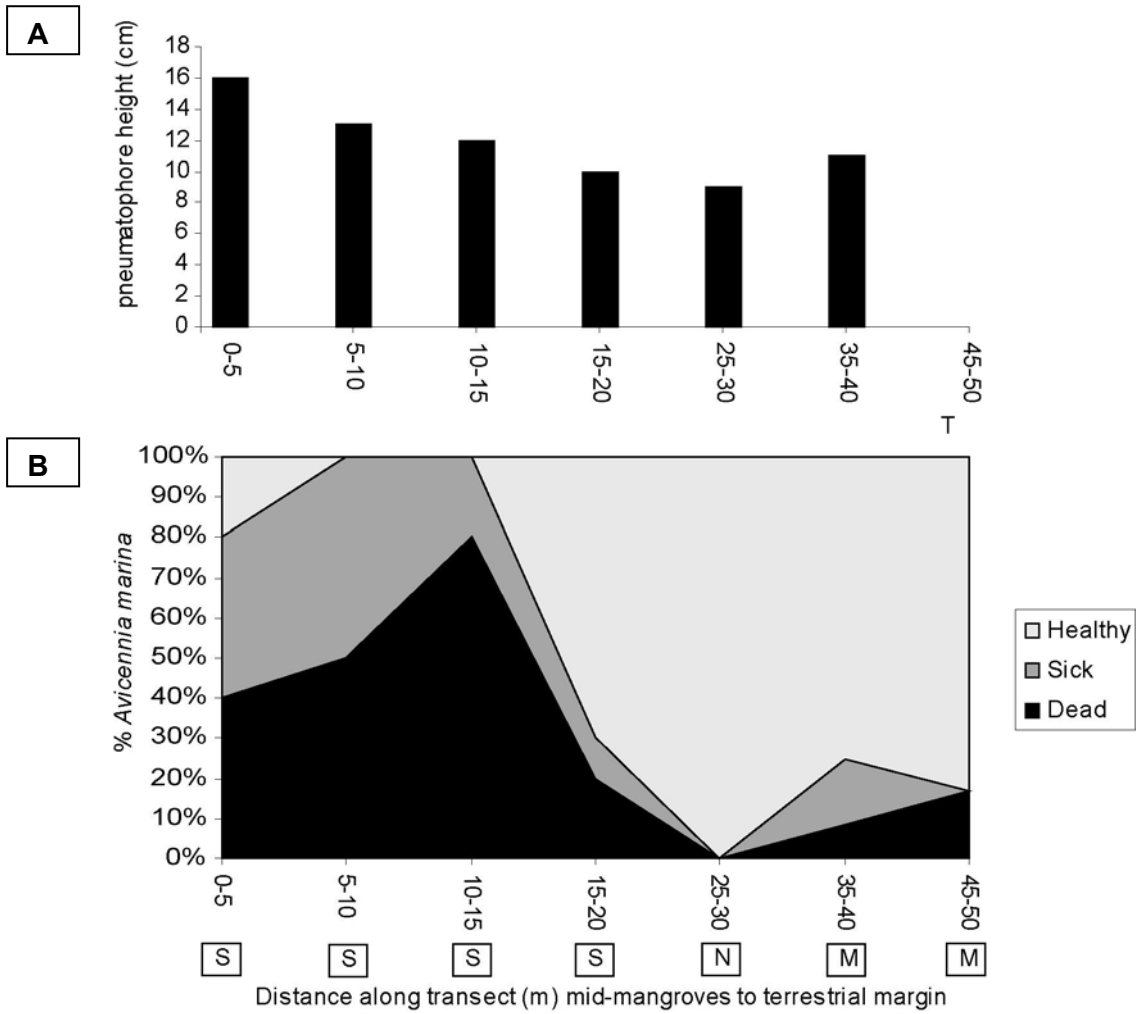

FIGURE 27: McCreadys Creek Transect 2 (MCT2). **(A)** Above-ground pneumatophore height (cm) along the transect **(B) C**ondition (healthy, sick or dead) of mangroves and the proportion of *A. marina* was represented in plots along the transect, which was run from the creek to the terrestrial margin (T) of the mangroves. *A. marina* mangroves were classified as having no dieback (N), moderate (M) or severe (S) dieback based upon their condition in the plot. For information on the location of the transect, refer to Figure 4.

Barnes Creek Transect 1 (BT1)

BT1 commenced at the water's edge of Barnes Creek, where there was a dead fringe of *A. marina*. There was notable erosion of the foreshore, where secondary cable roots of *A. marina* were set down, but primary cable roots were exposed. Forked pneumatophores were present along the transect as well as some sediment burial of the pneumatophores. Patterns of dieback were variable along the transect (Figure 28). This transect was dominated by *A. marina* and *R. stylosa*.

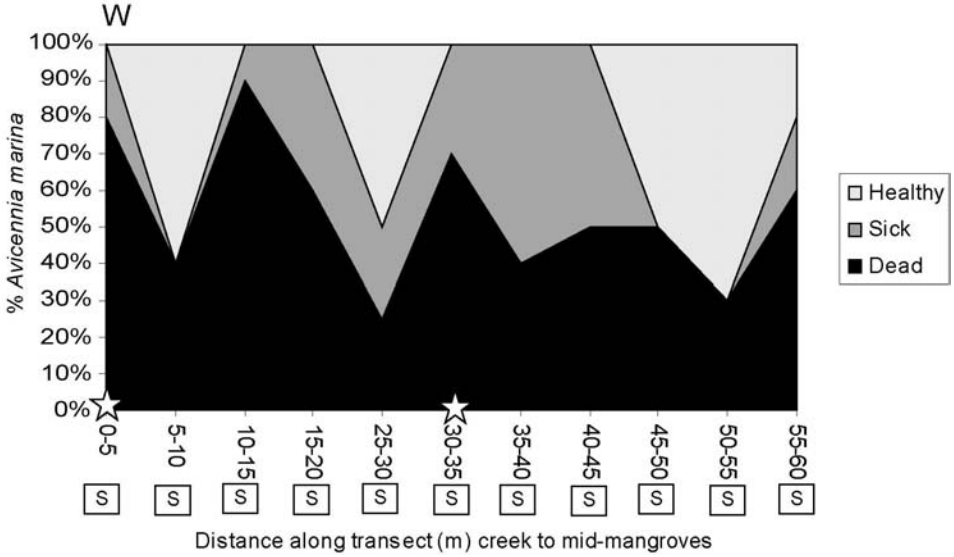

FIGURE 28: Transect 1 (BT1) in Barnes Creek was run from the water's edge (W) to the mid-mangroves. Condition (healthy, sick or dead) and proportion of *A. marina* was represented in plots along the transect. *A. marina* mangroves were classified as having no dieback (N), moderate (M) or severe (S) dieback based upon their condition in the plot. Sample for herbicide analysis (star) was taken from the mangrove core water (Table 15). For location of the transect, refer to Figure 5.

Barnes Creek Transect 2 (BT2)

This transect commenced at the water's edge on the southern side of Barnes Creek, This transect was dominated by *A. marina* and *R. stylosa.* As the transect progressed from the water's edge towards inland, the proportion of unhealthy (sick) and dead *A. marina* trees decreased (Figure 29). This transect was dominated by *A. marina* and *R. stylosa*.

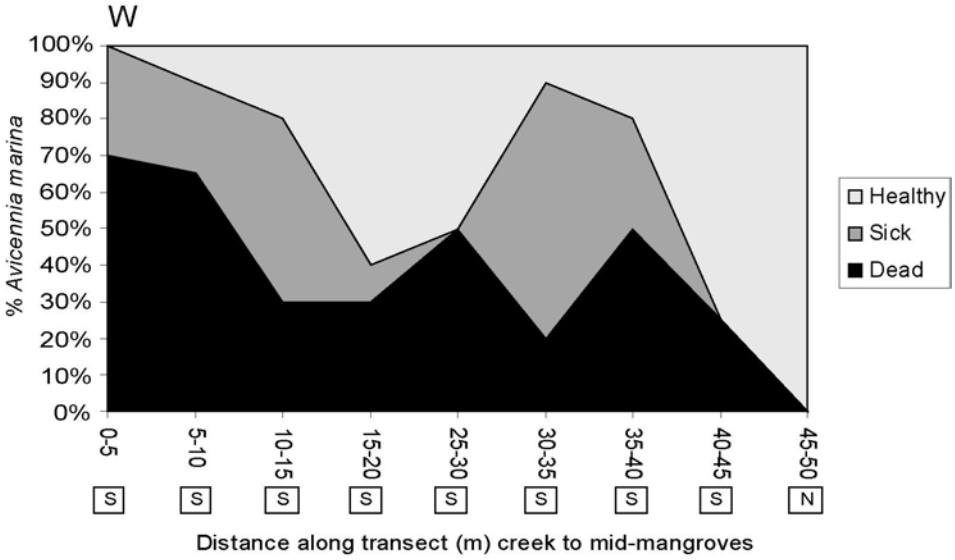

FIGURE 29: Transect 2 (BT2) in Barnes Creek was run from the water's edge (W) to the mid-mangroves. Condition (healthy, sick or dead) and proportion of *A. marina* was represented in plots along the transect. *A. marina* mangroves were classified as having no dieback (N), moderate (M) or severe (S) dieback based upon their condition in the plot. For location of the transect, refer to Figure 5.

Barnes Creek Transect 3 and 4 (BT3 and BT4)

These two transects were a continuation of each other. This transect began on the water's edge and headed landward towards the mangrove plot BH1. As with BT1, there was erosion of foreshore and cable roots were exposed in areas along the transect. The health of *A. marina* trees increased landward (Figure 30). The transect was dominated by *A. marina*, *A. corniculatum* and *R. stylosa*.

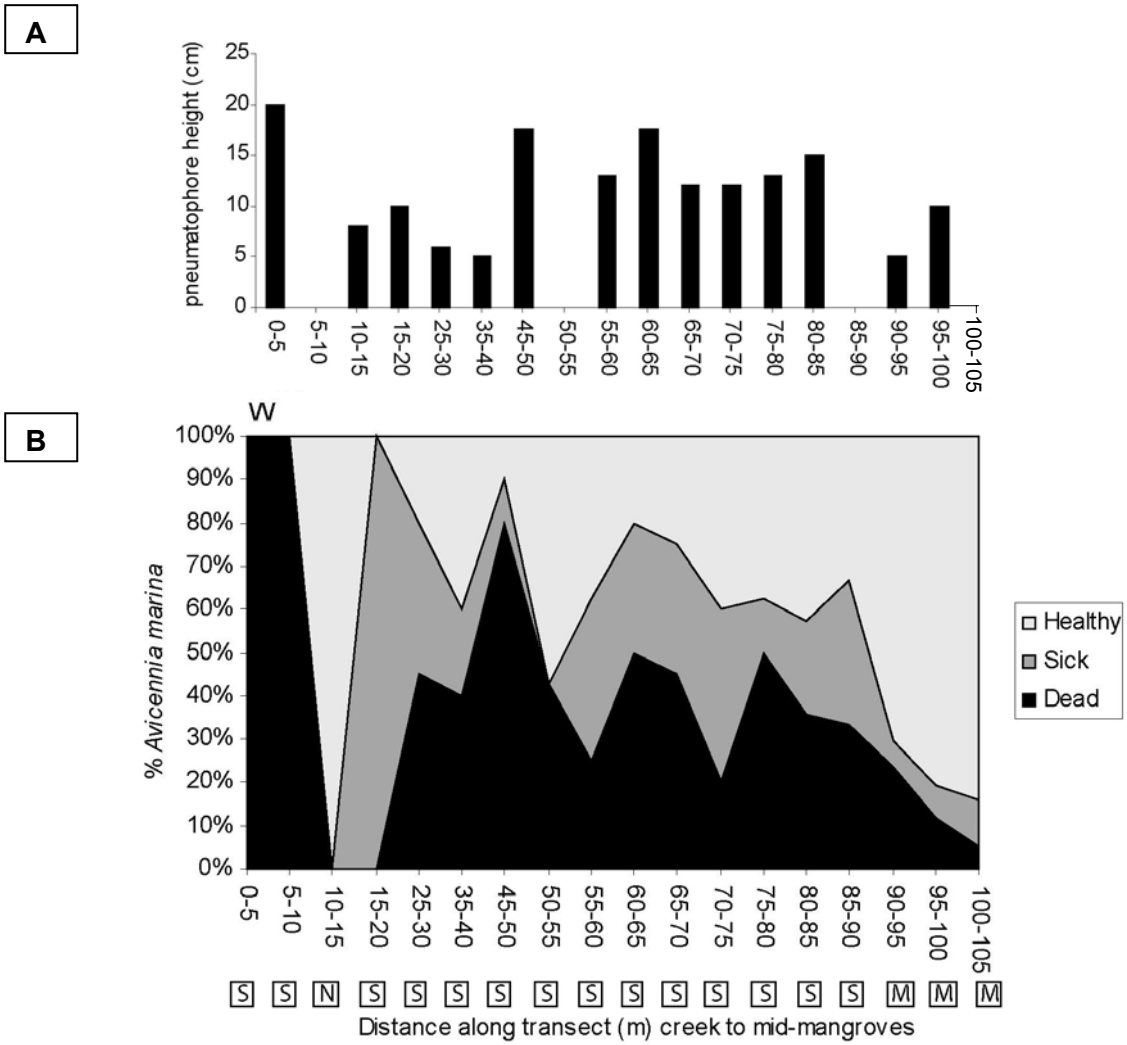

FIGURE 30: Barnes Creek transect 3 and 4 (BT3 and BT4) were combined. The transect was run from water's edge (W) to mid-mangroves. The condition (healthy, sick or dead) of mangroves and the proportion of *A. marina* trees were represented in plots along the transect. *A. marina* mangroves along each point in the transect were classified as having no dieback (N), moderate (M) or severe (S) dieback based upon their condition in the plot. For information on the location of the transect, refer to Figure 5.

Bakers Creek Transect 1 (BCT1)

BCT1 began mid-mangroves, close to Bakers Creek and headed towards a cane drain (near a bund wall). The health of *A. marina* decreased at both the seaward edge and the landward edge with a small patch of living *A. marina* in between and the entire transect was classified as severe (Figure 31). The dominant species were *A. marina*, *C. australis* and *Bruguiera exaristata*

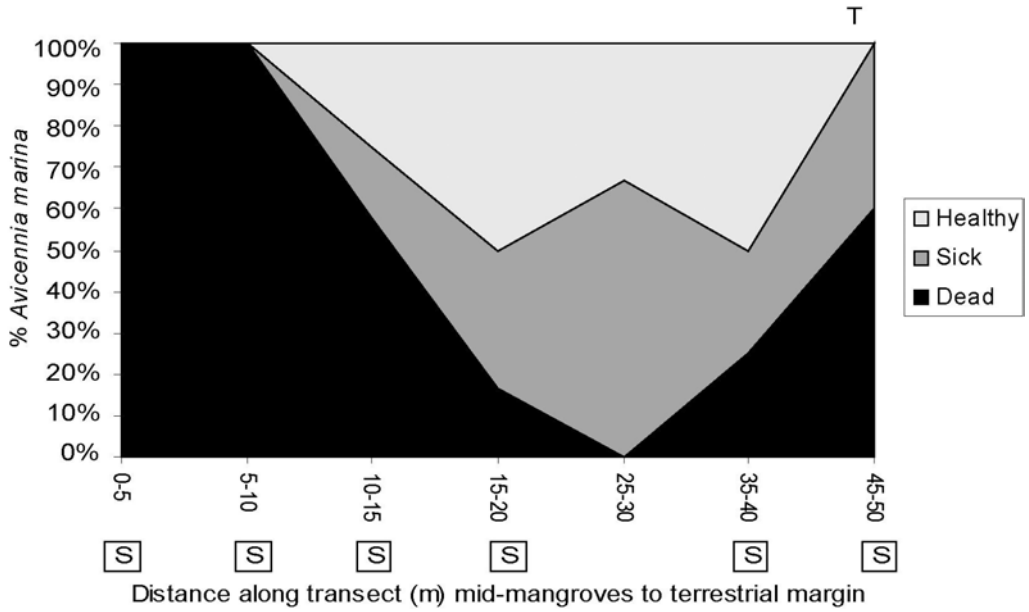

FIGURE 31: Bakers Creek Transect 1 (BCT1) was run from mid-mangroves to the terrestrial margin (T). Condition (healthy, sick or dead) and proportion of *A. marina* was represented in plots along the transect. *A. marina* was classified as having no dieback (N), moderate (M) or severe (S) dieback based upon their condition in the plot. For location of the transect, refer to Figure 6.

Bakers Creek Transect 2 (BCT2)

BCT2 began mid-mangroves close to a severe patch of dieback, and ended at terrestrial edge of the mangroves. The health of mangrove trees in this area was patchy (Figure 32). The dominant species were *A. marina, C. australis, B. exaristata* and *Aegialitis annulata*.

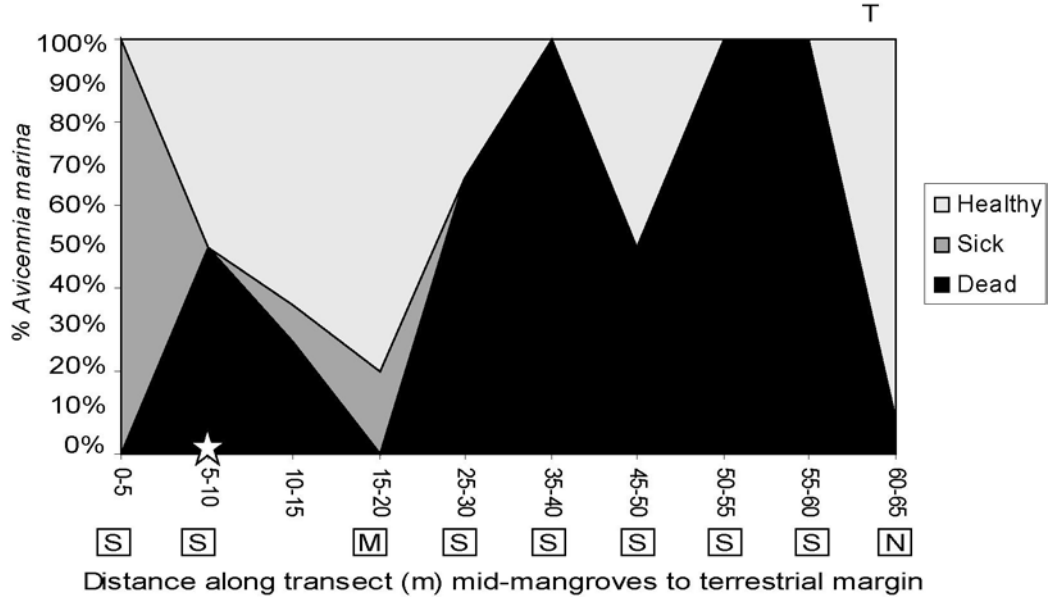

FIGURE 32: Bakers Creek transect 2 (BCT2) was run from mid-mangrove to the terrestrial margin (T) of the mangroves. Condition (healthy, sick or dead) and proportion of *A. marina* was represented in plots along the transect. *A. marina* was classified as having none (N), moderate (M) or severe (S) dieback based upon their condition in the plot. A sample for herbicide analysis (star) was taken from the mangrove core water (Table 15). For location of the transect, refer to Figure 6.

Plant Response Parameters – *A. marina*

Photosynthetic Activity

A. marina mangroves in Barnes Creek had lower maximum potential quantum yield (F_v/F_m) values for 3 out of the 4 plots where PAM readings were taken (Figure 33). Levels of F_v/F_m were above 0.75 in most plots for *A. marina*, which is considered to be the average range for healthy plants (0.75 –0.85).

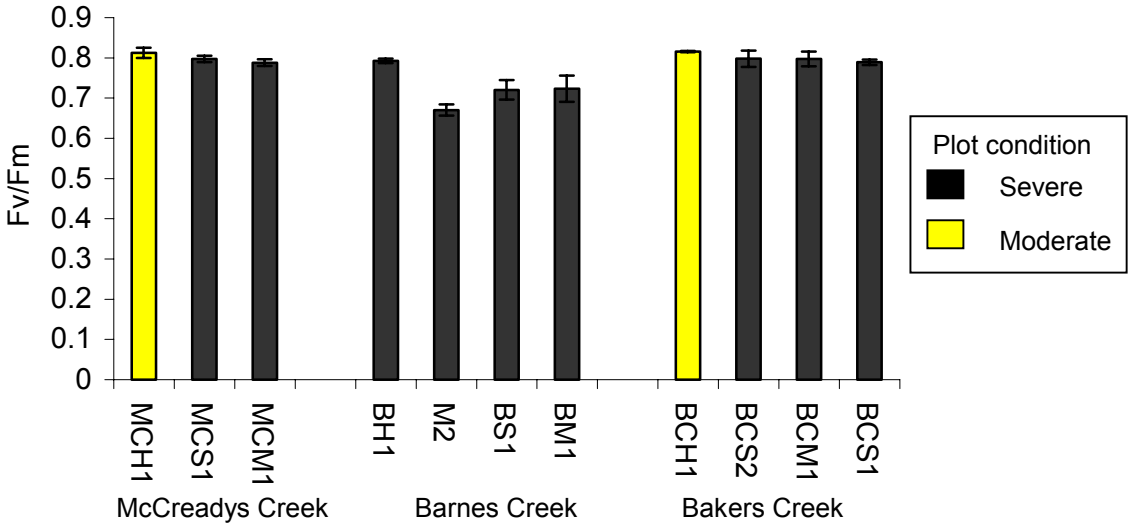

FIGURE 33: Maximum potential quantum yield (F_v/F_m) of *A. marina* trees. Moderate plots had 15 –30 % and severe plots had >30% of *A. marina* trees as sick or dead. Refer to Figures 4, 5 and 6 for site locations. Error bars are standard error terms for replicated measurements.

The PAM readings for all other species were between 0.75 – 0.85 for all sites at Bakers Creek and McCreadys Creek. In Barnes Creek at site BM1, *A. corniculatum* and *B. gymnorrhiza* were also showing signs of ill health (0.716 and 0.731 respectively). Furthermore at site M2, *C. australis* and *R. stylosa* had lower readings (0.582 and 0.692 respectively), however this is believed to be due to the high intensity of herbivory by insects on these particular species at this plot.

Leaf Chlorophyll

As expected, the healthy *A. marina* trees had normal and significantly higher levels of chlorophyll *a* in their leaves than the unhealthy trees (Figure 34).

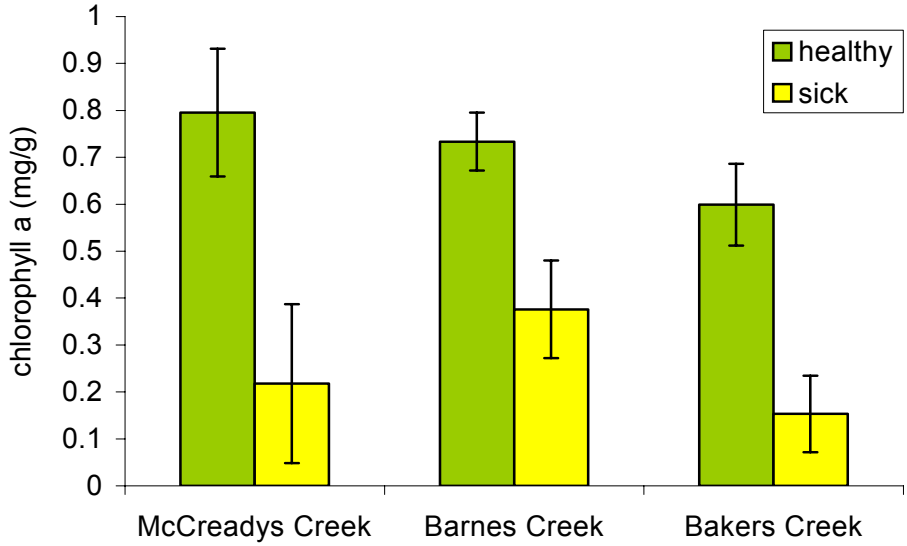

FIGURE 34: Chlorophyll *a* concentrations in *A. marina* mangrove leaves from McCreadys Creek, Barnes Creek and Bakers Creek. Error bars are standard error terms for replicated samples.

Physical Characteristics of Mangrove Sediments and Water

Organic Matter in Sediment

Each estuary differed in the amount of organic matter in the sediments (Figure 35). Bakers Creek had the highest amount of organic matter in the sediment, apart from BCS1, which is the plot where sedimentation was suspected to be occurring. Barnes Creek had low organic matter in the majority of plots, except for BH1.

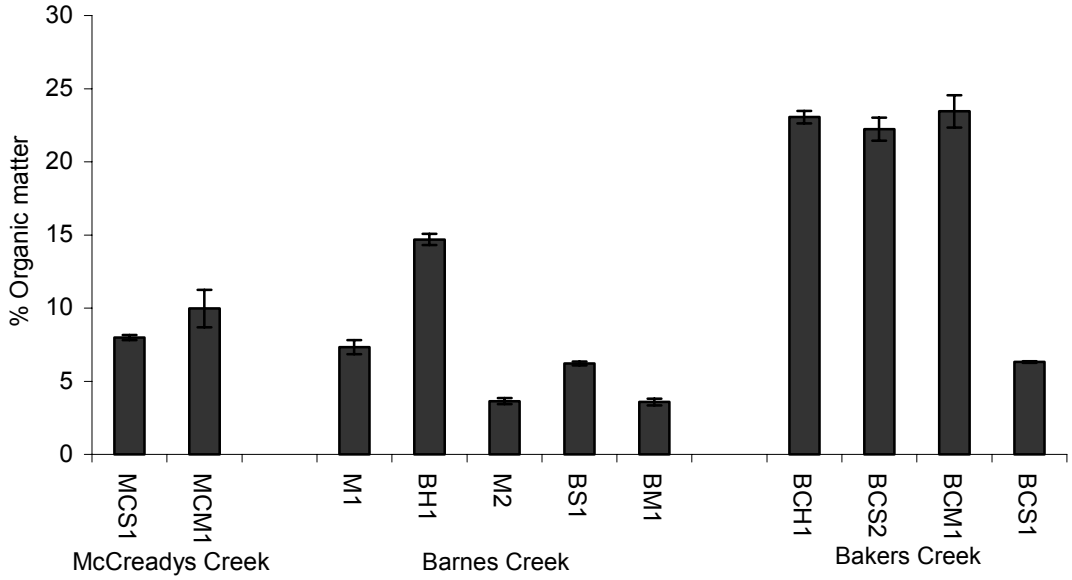

FIGURE 35: Percentage organic composition of the mangrove sediment in McCreadys Creek, Bakers Creek and Barnes Creek. Error bars are standard error terms for replicated samples. Refer to Figures 4,5 and 6 for site locations.

Mangrove Plot and River Water Quality

Measures of water quality were made from surface waters in tidal channels and from water that seeped into holes that were dug into mangrove sediment. Results for secchi depth, temperature, salinity, pH and dissolved oxygen are presented in Table 8.

Total Suspended Solids and Secchi Depth

Secchi depth is a measure of light attenuation and gives an indication of the level of total suspended solids in the water column. In the estuaries around Mackay, secchi depth generally increased further downstream, indicating increased turbidity in upstream sites. In the Pioneer River, secchi depth was low at sites PW1 and PW2 (0.5 m), which were upstream Barnes Creek where the water was observed to be murky and bubbling. Similarly, secchi readings were low upstream in McCreadys Creek, at site MCW1 (0.4 m). Bakers Creek had a variable secchi depth reading along the river, however determining a trend in the data was a problem as it was rapidly reaching low tide when sampling was conducted.

Temperature

Temperature was fairly constant within the rivers and also within the mangrove plots, and ranged between 24^{o}C and 26^{o}C.

Salinity
In all estuaries, surface water salinity increased further towards the mouth of the river. Salinity ranged between 35 to 38.7‰ in the Pioneer River, 25 to 37‰ in McCreadys Creek and 32.1 to 36.8‰ in Bakers Creek. Salinity remained close to seawater concentrations throughout Barnes Creek, Bakers Creek and McCreadys Creek. In the preliminary investigation (Duke *et al.,* 2001), lower salinities were found upstream in Barnes Creek (19.4‰) suggesting that freshwater runoff was greater at the time, that it was variable, and that it would influence mangrove growth seasonally. During the current investigation, there was very little rainfall to promote large volumes of freshwater runoff. Despite this, the flow was sufficient for sampling from agricultural drains.
Salinity levels were generally higher in the mangrove core water ranging from 42 to 54‰ in Barnes Creek, 39.1 to 41.8‰ in McCreadys Creek and 31.5 to 51.8‰ in Bakers Creek.

pH
All measured pH levels were within ANZECC (2000) guideline values (7.0 – 8.5) for all rivers tested, except for BCW1 at Bakers Creek, which was moderately alkaline with a pH of 8.8.

Dissolved Oxygen
The dissolved oxygen levels were below the guideline levels for slightly disturbed ecosystems in tropical Australia (80 – 120%) in all McCreadys Creek water sampling sites as well as one site within the Pioneer River (ANZECC, 2000). Levels of Dissolved Oxygen were within the ANZECC guideline values for Bakers Creek. Values for McCreadys Creek were all below the lower limit, especially MCW2, which was 54.9%. High levels of nutrients may cause low oxygen levels as a result of algal blooms, however Chlorophyll *a* levels in McCreadys Creek were lower than the Pioneer and Bakers Creek levels. These low values indicate some water quality problems, which need to be investigated further.

TABLE 8: Water quality parameters for the Pioneer River, McCreadys Creek and Bakers Creek. River sites are displayed as upstream to downstream. Refer to Figures 4, 5 and 6 for the location of the sites. A blank entry means that data was not collected. + indicates secchi disk hit the river bottom.

Site Name	Code	Secchi (m)	Temp. (°C)	Salin. (‰)	pH	DO (% sat)	Redox (mv)	Comment
River Sites								
Pioneer R	PW1	0.5	26.3	35		86.3	94.6	bubbles
Pioneer R	PW2	0.5	26.8	35		67.6	121.5	
Pioneer R	PW3	1.7+	26.6	36.8		84	106.9	
Pioneer R	PW4	1.8	26.8	38		89	33	
Pioneer R	PW5	2.2	26.4	38.7		91.9	60.2	
McCreadys Ck	MCW1	0.4	25	25	7.6	76.3		moderate
McCreadys Ck	MCW2	0.8	25.5	34.4	7.5	54.9		
McCreadys Ck	MCW3	1.1	25.8	36.4	7.83	73.2		
McCreadys Ck	MCW5	1.3+	26	37	7.92	77.9		
McCreadys Ck	MCW4	1.5+	26	37.4	7.97	79		no dieback
Bakers Ck	BCW5	0	26.1	32.1	7.9	101.3		
Bakers Ck	BCW4	1.1	24.6	36.4	7.8	89.2		
Bakers Ck	BCW3	0.2	24.5	36.2	8	88.1		
Bakers Ck	BCW2	1+	24.3	36.8	8.06	86.5		
Bakers Ck	BCW1	0.7	24.1	36.6	8.8	87.9		
Mangrove Core Water								
Barnes Ck	BH1	n/a	25.8	42.6	7.02	3.5	7.1	
Barnes Ck	BM1	n/a	26.8	42	6.18	19.6	52.6	
Barnes Ck	BM1	n/a	25.5	42	5.89	10	72.5	
Barnes Ck	BM1	n/a	25.5	43	6.35		46	
Barnes Ck	BS1	n/a	25.1	48	6.69	10.8	23.4	
Barnes Ck	M2	n/a	24.9	54	6.55		30.1	
McCreadys Ck	MCH1	n/a	23	41.8	6.46	1.8		
McCreadys Ck	MCM1	n/a	25	41.6	6.4	0.1		
McCreadys Ck	MCS1	n/a	25	39.1	6.69	1.5		
Bakers Ck	BCH1	n/a	23.8	51.8	6.05	2.94	37.5	
Bakers Ck	BCM1	n/a	24.3	49.2	6.43	9.71	20.6	
Bakers Ck	BCS1	n/a	24	35.9	6.2	11.6	35	
Bakers Ck	BCS2	n/a	22.3	40.1	6.57	2.6	8	

Benthic Microalgae (BMA)

Within each estuary, the total chlorophyll of benthic microalgae increased with amount of unhealthy and dead trees in the plots, except in the site BCS1 (Figure 36 a and b). There was no relationship between herbicide concentration in the sediment and BMA abundance.

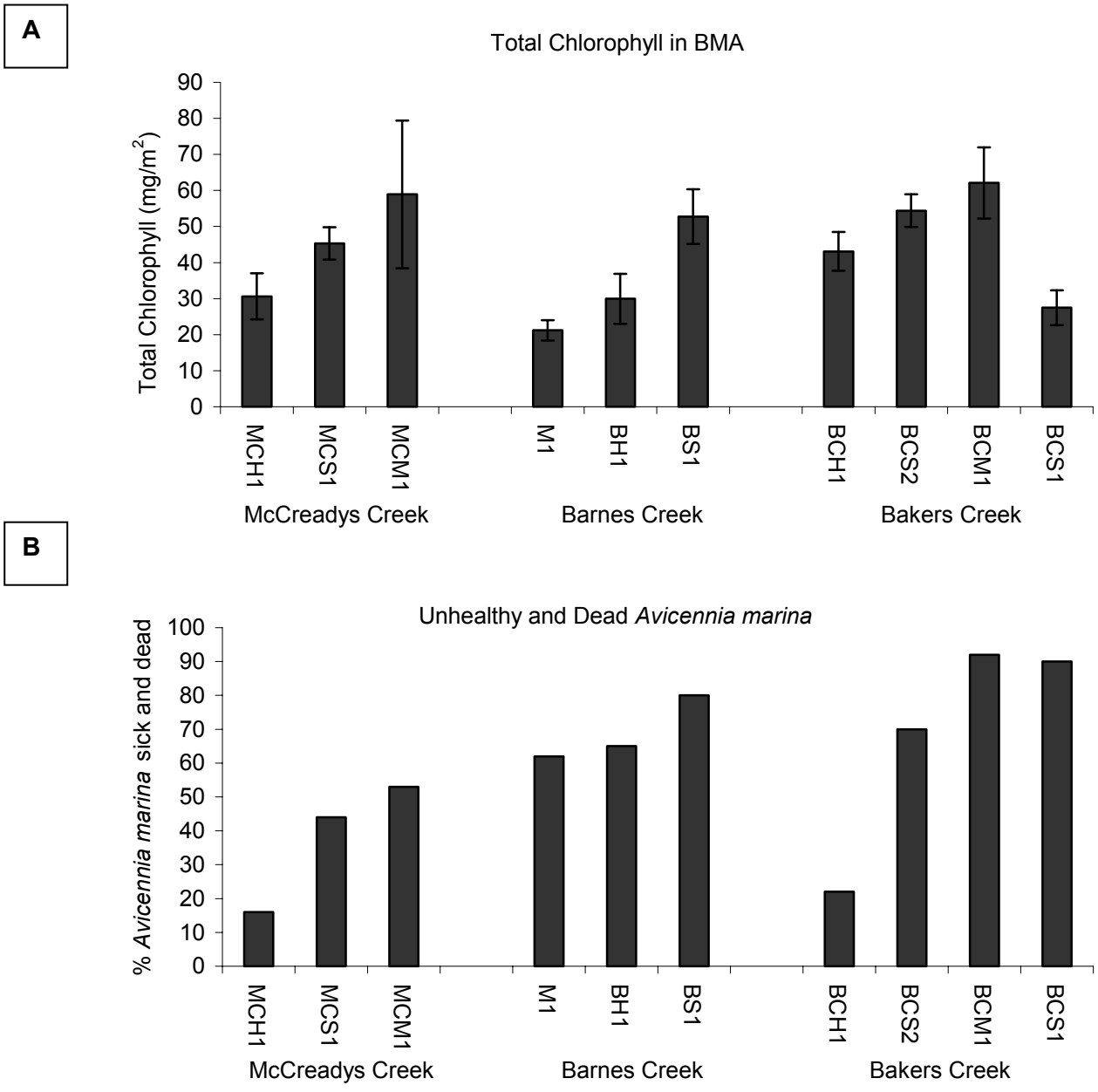

FIGURE 36: (A) Total chlorophyll in sites throughout the Mackay region **(B)** % unhealthy (sick) and dead *A. marina* trees in mangrove plots. Refer to Figures 4,5 and 6 for site location.

Likely Agents Causing Mangrove Dieback

Heavy Metals

Sediment

Concentrations of heavy metals in mangrove sediments varied between sites for all estuaries (Table 9). Of all estuaries, Bakers Creek had the highest averaged concentrations of As, Cr, Cu, Fe, Mn, Ni and Zn. Barnes Creek had the highest average concentration of Pb. Hg and Cd concentrations were below the detection limit. The highest overall heavy metal concentrations were seen at the Bakers Creek site, BCH1 for all heavy metals except for Mn, which was highest at site M1 within Barnes Creek.

In the sediment none of the values for As, Cr, Cu, Pb, Zn, Hg or Cd exceeded the interim sediment quality guideline (ISQG) low value in the ANZECC guidelines (Appendix 16). ANZECC guideline values were not available for Fe, Mn or Ni in sediments.

A one-way ANOVA (95% confidence) was conducted on all heavy metals using STATISTICA software, V6, to determine whether there was any significant difference between estuaries for any of the elements tested. Results of this ANOVA confirm that there was no significant difference.

Water

Concentrations of As, Ni, Zn, Hg and Cd were below the detection limit in all samples (Table 9). Of the remaining heavy metals (Fe, Mn and Pb), there was a significant correlation between concentrations in the water and in the sediment for Fe ($p<0.02$, $r^2=0.4185$, $n=10$) and for Mn ($p<0.05$, $r^2=0.4426$, $n=10$). There was no significant relationship for Pb.

A one-way ANOVA (95% confidence) was conducted on all heavy metals using STATISTICA software, V6, to determine whether there was any significant difference between estuaries for any of the elements tested. Results of this ANOVA confirm that there was no significant difference.

Leaves

Concentrations of heavy metals in mangrove leaves are presented in Table 10. A one-way ANOVA was used to find if there was a significantly different concentration of elements in *A. marina* leaves from different estuaries. Due to insufficient leaf samples, ANOVA could not be conducted on unhealthy leaves, so comparisons were made with healthy leaves only. A significant F-value was found using one-way ANOVA for Zn, so a Least Significant Different (LSD) Test was used to determine differences between means. The concentration of Zn in McCreadys Creek was significantly higher ($p<0.05$) in healthy leaves of *A. marina* than in Barnes and Bakers Creek.

In order to determine whether there was a difference in concentration of heavy metals between healthy and unhealthy *A. marina* leaves, a t-test was conducted for each element detected in mangrove leaves. In the leaves of *A. marina*, there was significantly more Cu ($p<0.01$), Zn ($p<0.005$) and Cl ($p<0.01$) in healthy than in unhealthy leaves. On the contrary, there was more Ca ($p<0.05$), Mn ($p<0.02$) and Mg ($p<0.01$) in unhealthy than in healthy leaves of *A. marina*. There was no significant difference between concentrations of K, Na, Cr and Fe in healthy and unhealthy leaves.

TABLE 9: Concentrations of heavy metals in mangrove sediment and core water. Samples were from McCreadys Creek, Bakers Creek and Barnes Creek (Pioneer River). For location of sites, refer to Figures 4, 5 and 6.

SEDIMENT		Detected heavy metals (mg/kg)									
Site location	**Site Code**	**As**	**Cr**	**Cu**	**Fe**	**Mn**	**Ni**	**Pb**	**Zn**	**Hg**	**Cd**
McCreadys Creek	MCH1	9	20	16	17300	100	9.9	7	30	<0.2	<1
McCreadys Creek	MCS1	4	13	8	9900	71	5.6	5	15	<0.2	<1
McCreadys Creek	MCM1	4	10	8	8800	63	4.9	3	13	<0.2	<1
Barnes Creek	M1	6	22	26	22700	250	11	11	44	<0.2	<1
Barnes Creek	BH1	5	15	24	14700	98	8	13	44	<0.2	<1
Barnes Creek	M2	2	8	10	6500	84	3.6	7	21	<0.2	<1
Barnes Creek	BS1	6	15	20	15500	110	7.6	7	28	<0.2	<1
Bakers Creek	BCH1	11	34	33	29000	180	16	13	51	<0.2	<1
Bakers Creek	BCS2	9	30	29	24700	120	15	11	44	<0.2	<1
Bakers Creek	BCM1	8	26	25	23000	120	13	1	39	<0.2	<1
Bakers Creek	BCS1	4	15	14	13000	180	6.9	6	21	<0.2	<1
Detection Limit		2	0.2	1	0.4	0.06	0.2	1	0.1	0.2	1

WATER		Detected heavy metals (µg/L)									
Site location	**Site Code**	**As**	**Cr**	**Cu**	**Fe**	**Mn**	**Ni**	**Pb**	**Zn**	**Hg**	**Cd**
McCreadys Creek	MCH1	<20	2	<5	5200	250	<5	36	<20	<10	<1
McCreadys Creek	MCS1	<20	<2	<5	1500	75	<5	37	<20	<10	<1
McCreadys Creek	MCM1	<20	<2	<5	1400	86	<5	35	<20	<10	<1
Barnes Creek	M1	<20	<2	<5	4000	320	<5	35	<20	<10	<1
Barnes Creek	BH1	<20	2	<5	4800	140	<5	47	<20	<10	<1
Barnes Creek	M2	<20	<2	<5	1500	95	<5	36	<20	<10	<1
Barnes Creek	BS1	<20	2	6	5600	300	<5	37	<20	<10	<1
Bakers Creek	BCH1	<20	<2	<5	6200	320	<5	36	<20	<10	<1
Bakers Creek	BCS2	<20	<2	<5	4200	270	<5	33	<20	<10	<1
Bakers Creek	BCM1	<20	<2	<5	2200	210	<5	30	<20	<10	<1
Bakers Creek	BCS1	<20	<2	<5	1600	690	<5	36	<20	<10	<1
Detection Limit		20	2	5	10	10	5	20	20	10	1

TABLE 10: Heavy metals in *A. marina* leaves from healthy and unhealthy (sick) leaves. Samples were taken from McCreadys, Barnes and Bakers Creek. For location of sites, refer to Figures 4, 5 and 6. Note: No data indicates no analysis was performed.

LEAVES		Detected heavy metals (mg/kg)									
		K		Na		Ca		Cr		Cu	
	Site code	Healthy	Unhealthy	Healthy	Unhealthy	Healthy	Unhealthy	Healthy	Unhealthy	Healthy	Unhealthy
McCreadys Creek	MCH1	13000	13800	23800	17400	4800	5200	7.2	25	8	2
McCreadys Creek	MCS1	9100	N/A	27200	N/A	4600	N/A	1.2	N/A	9	N/A
McCreadys Creek	MCM1	10000	12300	25000	15600	3300	5900	2.3	2.7	8	3
Barnes Creek	M1	9100	9100	15500	17500	4000	3400	1.6	1.4	11	12
Barnes Creek	BH1	7400	8600	20400	16700	5400	5700	1.6	1.2	10	7
Barnes Creek	M2	10300	N/A	25100	N/A	4500	N/A	0.4	N/A	7	N/A
Barnes Creek	BS1	9800	8100	27200	18200	5900	6700	2.3	4.2	6	4
Barnes Creek	BM1	10700	9600	19200	24300	3200	4500	20	20	14	5
Bakers Creek	BCH1	8000	N/A	14900	N/A	3600	N/A	1	N/A	6	N/A
Bakers Creek	BCS2	9400	N/A	19300	N/A	5100	N/A	0.8	N/A	6	N/A
Bakers Creek	BCM1	6400	6900	34300	23200	5700	7500	2.6	3	11	4
Bakers Creek	BCS1	6900	9500	20600	19200	3400	6400	6.5	9.9	9	6
Detection Limit		10		30		2		0.2		1	
		Fe		Mn		Mg		Zn		Cl	
	Site code	Healthy	Unhealthy	Healthy	Unhealthy	Healthy	Unhealthy	Healthy	Unhealthy	Healthy	Unhealthy
McCreadys Creek	MCH1	130	350	38	140	5200	13400	23	7.9		39000
McCreadys Creek	MCS1	68	N/A	23	N/A	3400	N/A	17	N/A	89000	N/A
McCreadys Creek	MCM1	57	110	19	120	3600	9600	21	9.7	84000	32000
Barnes Creek	M1	120	70	130	59	8200	5100	13	12	40700	44100
Barnes Creek	BH1	210	150	41	65	5400	8100	9.1	8.6	57500	31500
Barnes Creek	M2	120	N/A	60	N/A	8900	N/A	13	N/A	72000	N/A
Barnes Creek	BS1	1000	1200	100	180	10200	11700	13	9.1	50200	55300
Barnes Creek	BM1	380	640	48	68	7300	10000	18	8.5		
Bakers Creek	BCH1	170	N/A	95	N/A	11900	N/A	10	N/A	30400	N/A
Bakers Creek	BCS2	110	N/A	88	N/A	9100	N/A	13	N/A	46500	N/A
Bakers Creek	BCM1	180	190	53	160	4200	13000	17	12	106000	45000
Bakers Creek	BCS1	140	230	86	210	7000	13300	14	11	64400	
Detection Limit		0.4		0.06		0.9		0.1		5	

Nutrients

Core Water Nutrients

Nitrogen and phosphorus concentrations detected in the mangrove core water are displayed in Table 11. Concentrations of nitrogen and phosphorus at each site were variable. Nitrite (NO_2) was below the detection limit (0.002) in all the plots, therefore the reported nitrogen oxide levels comprised mainly of nitrate (NO_3).

Duke *et al.,* (2001) found a significant correlation ($p<0.001$) between total nitrogen and total phosphorus in creek and core hole water. Data from the current investigation was incorporated with the results from the preliminary investigation, and the result was quite significant (r^2 =0.7526) with log transformed data (Duke *et al.,* 2001) – see Figure 37. This indicates that the concentrations of nitrogen and phosphorus in the Mackay region are strongly related.

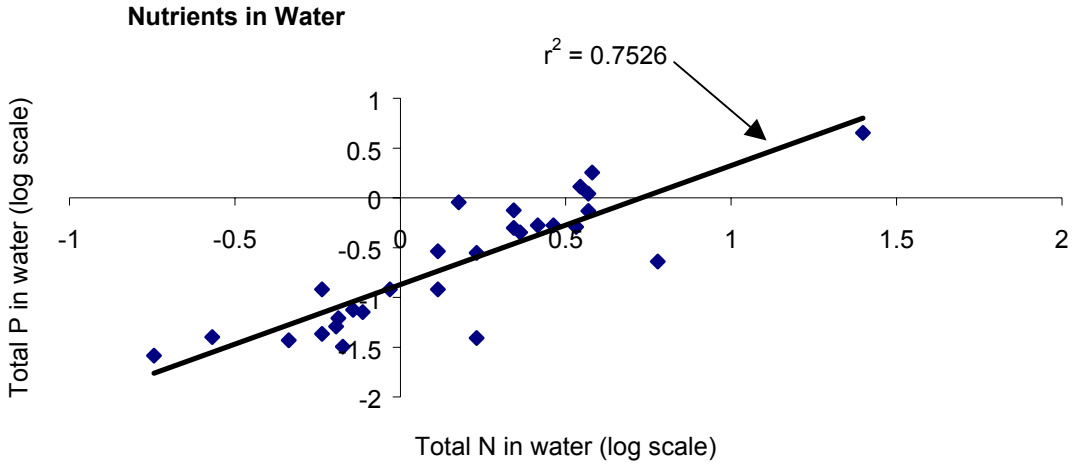

FIGURE 37: Significant correlation (r^2 =0.7525, n =28, P<0.001) between total nitrogen and total phosphorus in the core water from all plots in the Mackay region (including data from Duke *et al.,* 2001). Data was log transformed.

Existing guideline values for nutrients in waters do not specify core water, however ANZECC (2000) provide guidelines for nutrients in lowland QLD tropical waters (Appendix 17). Polluted water bodies will have significant stores of N and P in the sediments, therefore nutrient concentrations in the sediment corewater will not necessarily mirror concentrations in the water column (ANZECC, 2000). Nevertheless, these guidelines will be used in this report to give an indication of the ideal concentration of nutrients in the mangrove corewater in Mackay.

The geometric means of the data are within error terms of the arithmetic means, therefore all calculations are of arithmetic means, due to lack of sufficient data to justify using geometric means.

One-way ANOVA (95% confidence) was carried out for each nutrient to determine whether there was a significant difference between estuaries using STATISTICA v6. There was no significant difference between sites for any of the nutrients.

TABLE 11: Nitrogen and phosphorus concentrations of mangrove core water from mangrove plots in Mackay (mg/L). Refer to Figures 4, 5 and 6 for location of sites.

		Phosphorus			Nitrogen			
Site	**Code**	**Total mg/L as P**	**Total Diss. mg/L as P**	**Filt. Reac. mg/L as P**	**Ammonia (NH_3) mg/L as N**	**Oxides ($N0_2$ + $N0_3$) mg/L as N**	**Total Diss. mg/L as N**	**Total mg/L as N**
McCreadys Ck	MCH1	0.74	0.01	<0.002	0.29	0.011	0.92	3.7
McCreadys Ck	MCS1	1.1	0.2	0.16	0.91	0.007	1.5	3.7
McCreadys Ck	MCM1	0.51	0.021	0.007	0.095	0.013	0.55	3.4
Barnes Ck	M1	0.29	0.029	<0.002	0.081	0.005	0.47	1.3
Barnes Ck	BH1	1.3	0.058	0.043	0.14	0.011	1.0	3.5
Barnes Ck	M2	0.75	0.56	0.46	0.15	0.017	1.2	2.2
Barnes Ck	BS1	0.5	0.007	<0.002	0.68	0.009	1.1	2.2
Bakers Ck	BCH1	0.53	0.015	<0.002	0.064	0.003	0.72	2.9
Bakers Ck	BCS2	1.8	0.22	0.18	2.1	0.008	3.0	3.8
Bakers Ck	BCM1	2.3	0.15	0.12	1.1	0.007	1.7	6.0
Bakers Ck	BCS1	0.53	0.024	<0.002	0.44	0.005	0.88	2.6
Detection limit		0.002	0.002	0.002	0.002	0.002	0.02	0.02

McCreadys Creek

Every sample exceeded ANZECC (2000) guidelines for Total N. Based on arithmetic mean values, McCreadys Creek sites collectively had almost the same total N as the sites from Bakers Creek (3.6 and 3.8mg/L respectively). The mean value of Total Dissolved N was about the same in McCreadys Creek as in Barnes Creek (0.99 and 0.94 mg/L respectively). The mean of oxides of N in McCreadys Creek was the same as Barnes Creek (0.01mg/L for both estuaries), however these values were almost double of those detected in Bakers Creek. The ANZECC (2000) guidelines for ammonium/ammonia concentration (as N) were exceeded in all samples and the mean of ammonia concentrations was intermediate (0.26mg/L).

All samples at McCreadys Creek exceeded the ANZECC (2000) guidelines for Total P. McCreadys Creek had an intermediate mean for total P (0.78). The mean of total dissolved P concentrations was lowest at McCreadys Creek (0.08mg/L). Two sites exceeded FRP at McCreadys Creek, particularly MCS1.

McCreadys Creek had the highest TN: TP ratio (4.6:1 arithmetic)

Barnes Creek

Barnes Creek had the lowest mean Total N (2.3mg/L), however every sample in Barnes Creek exceeded the ANZECC (2000) guidelines for Total N. The mean of Total Dissolved N was about the same in Barnes Creek as in McCreadys Creek (0.94 and 0.99mg/L respectively). The mean of oxides of N in Barnes Creek was the same as McCreadys Creek (0.01mg/L for both estuaries). The mean for ammonia was also lowest at Barnes Creek (0.26mg/L) and again the ANZECC (2000) guidelines for ammonium/ammonia concentration (as N) were exceeded in all samples.

Every sample at Barnes Creek exceeded the ANZECC (2000) guidelines for Total P and the mean for Total P was lowest (0.71mg/L). The mean of total dissolved P concentrations was highest in Barnes Creek (0.16mg/L). Site M2 within Barnes Creek had the highest concentration of Total Dissolved P of all sites in all estuaries (0.56mg/L). FRP exceeded the ANZECC (2000) guidelines at 2 sites at Barnes Creek, especially M2, which had the highest concentration of all sites in all estuaries.

Barnes Creek had an intermediate TN: TP ratio (3.2:1 arithmetic).

Bakers Creek

Every sample exceeded ANZECC (2000) guidelines for Total N. Bakers Creek had almost the same mean for Total N as McCreadys Creek (3.8 and 3.6 mg/L respectively). The mean of Total Dissolved N was highest in Bakers Creek (1.5mg/L). Site BCS2 had the highest concentration of total dissolved N of all sites in all estuaries. The mean of N oxides was lowest in Bakers Creek (0.005mg/L), approximately half that detected in McCreadys Creek and Barnes Creek. The ANZECC guidelines for ammonium/ammonia concentration (as N) were exceeded in all samples. The mean for ammonia was highest at Bakers creek (0.93mg/L). Bakers Creek had two sites, BCS2 and BCM1, with extremely high ammonia concentrations.

The mean for Total P in Bakers Creek was highest of all 3 estuaries (1.29mg/L). Every sample exceeded the guidelines for TP, and site BCM1 had the highest reported level of Total P from all sites in all estuaries (2.3 mg/L). Bakers Creek had the highest mean for Total Dissolved P of all estuaries. FRP concentrations exceeded the ANZECC (2000) guidelines at two sites at Bakers Creek.

Bakers Creek had the lowest TN: TP ratio (3.0:1 arithmetic)

Leaf Nutrients

Table 12 displays the results for nitrogen (sewage-derived nitrogen and %N) and carbon (δ13C) values detected in *A. marina* leaves from McCreadys Creek, Barnes Creek and Bakers Creek.

TABLE 12: Nitrogen and Carbon content of unhealthy (sick) and healthy leaves of *A. marina* in McCreadys Creek, Bakers Creek and Barnes Creek. For location of sites, refer to Figures 4, 5 and 6. No data indicates that no analysis was performed.

Mangrove Leaves		Del N		%N		Del C	
Site	Code	Healthy	Sick	Healthy	Sick	Healthy	Sick
McCreadys Ck	MCH1	3.7	3.4	2.3	0.7	-27.4	-27.5
McCreadys Ck	MCS1	2.3		2.5		-28.2	
McCreadys Ck	MCM1	2.3	2.9	1.9	1.4	-28.6	-25.8
Barnes Ck	M1	7.5	7.6	1.7	1.5	-28.7	-27.8
Barnes Ck	BH1	4.4	5.1	1.7	1.6	-27.8	-27.4
Barnes Ck	M2	3.7		1.7		-28.6	
Barnes Ck	BS1	6.8	8.2	1.8	0.9	-28.5	-27.4
Barnes Ck	BM1	7.1	5.8	2	1.2	-27.7	-28
Bakers Ck	BCH1	5		1.6		-27.3	
Bakers Ck	BCS2	4.6		2.2		-25.8	
Bakers Ck	BCM1	4.2	4.7	2.7	0.8	-28.4	-25.8
Bakers Ck	BCS1	7.2	7.2	1.6	0.9	-28.1	-27.4

Nitrogen Content

%N – There was a greater percentage of nitrogen in healthy than sick leaves (Table 12). There was a correlation between the % nitrogen in *A. marina* leaves and the concentration of both total Nitrogen (Figure 38) and Ammonia (Figure 39) in the mangrove sediment core water.

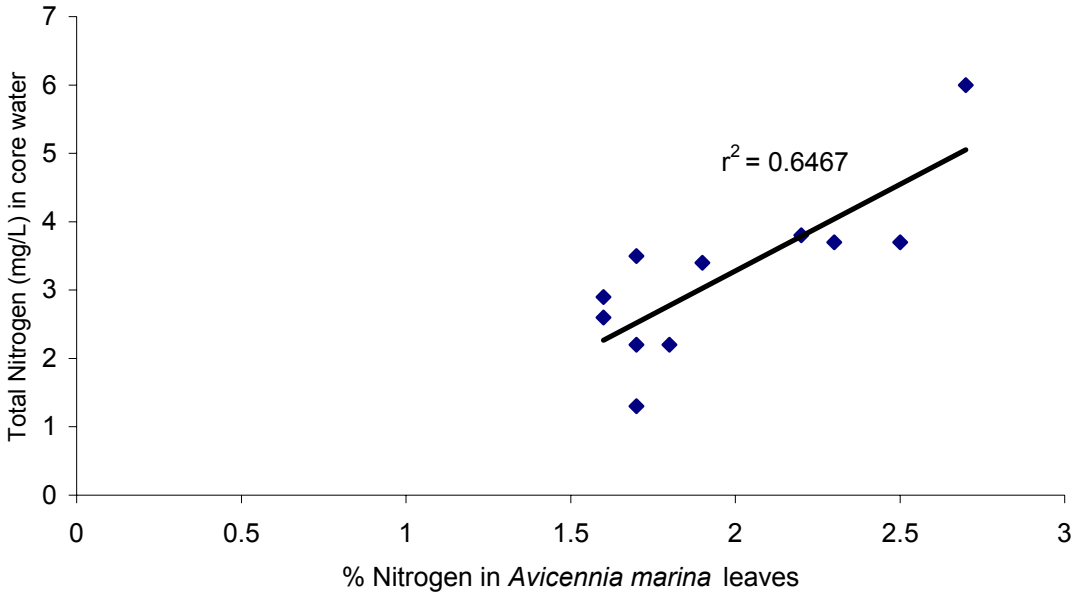

FIGURE 38: Significant correlation (r^2=0.6467, n=11, p<0.002) between the % nitrogen in *A. marina* leaves and total nitrogen in the core water.

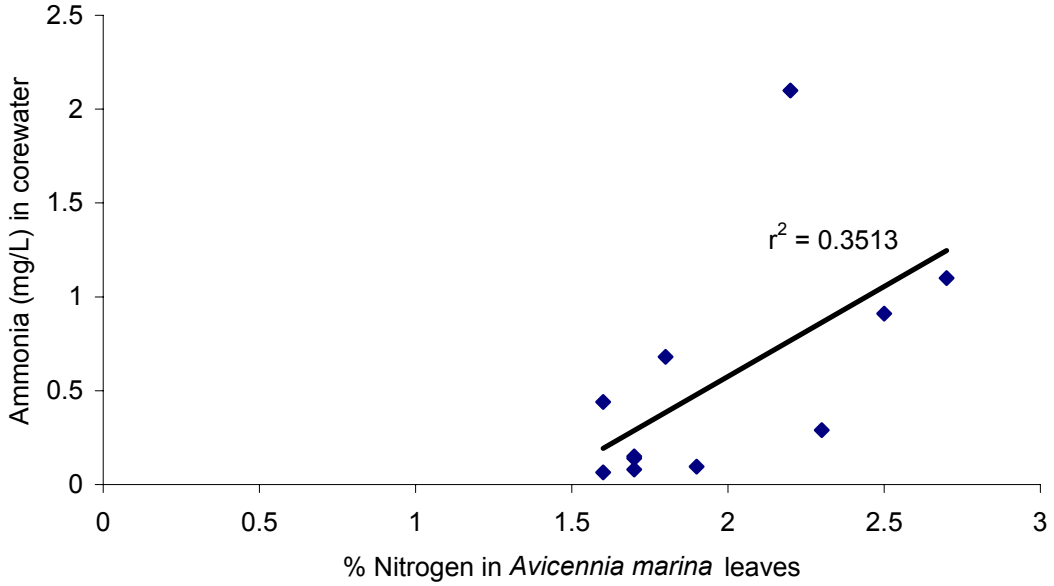

FIGURE 39: Significant correlation (r^2=0.3513, n-11, p<0.05) between the % nitrogen in *A. marina* leaves and the concentration of ammonia (mg/L) in the mangrove sediment core water.

Sewage-Derived Nitrogen (δ15N) - The highest levels of $\delta^{15}N$ (Table 12) were recorded in Barnes Creek, with an average value of 5.9, while in Bakers Creek there was one site immediately downstream from the meatworks and an aquaculture facility that also had elevated levels of $\delta^{15}N$. The average $\delta^{15}N$ value for Bakers Creek was also quite high (5.25), while McCreadys Creek had a lower average $\delta^{15}N$ value (2.8). There were no differences in sewage-derived nitrogen uptake between healthy and unhealthy trees, as seen in the preliminary investigation (Duke *et al.,* 2001). Values were not as high as detected in the STP creek in the preliminary investigation, where levels ranged from between 13.51 to 15.97 in a number of mangrove species.

A one-way ANOVA was conducted using STATISTICA v6.0 software, to determine whether there was any difference in $\delta^{15}N$ between sites in healthy mangrove leaves. A significant F-value existed between sites, so a LSD Test was used to determine which sites were different. McCreadys Creek had a significantly lower average $\delta^{15}N$ value (2.8) to Barnes Creek ($p<0.05$) but not to Bakers Creek.

In comparing plots M1 and M2 in both 2000 and 2002, the values for $\delta^{15}N$ were similar with values of 7.72 and 2.46 respectively in healthy *A. marina* plants in the previous investigation (Duke *et al.,* 2001) and 7.5 and 3.7 respectively in the current investigation. Plot M2 (the plot showing signs of recovery) had the lowest level of $\delta^{15}N$ in mangrove leaves for Barnes Creek. All plots in McCreadys Creek had the lowest values of $\delta^{15}N$ in mangrove leaves.

Although there was a relationship between oxides of N in the water and the $\delta^{15}N$ of *A. marina* leaves in the preliminary investigation, there was no relationship found in this investigation, neither was there a significant relationship if the data was pooled together.

Carbon 13

Carbon 13 values were significantly higher in the unhealthy leaves than the healthy leaves in all estuaries (Figure 40). Healthy or unhealthy leaves did not have significantly different values using a one-way ANOVA for Carbon 13 between estuaries.

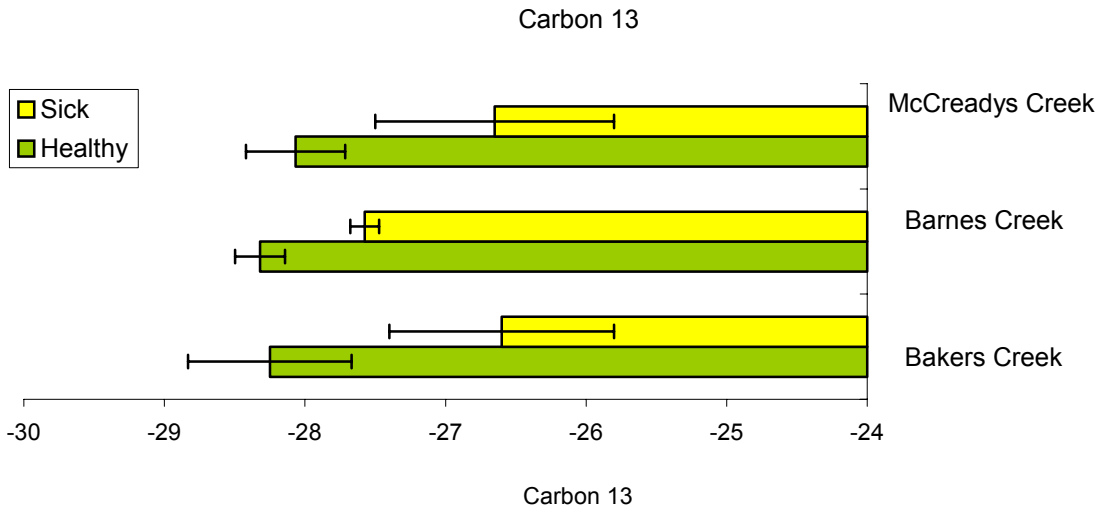

FIGURE 40: Carbon 13 in *A. marina* from healthy and unhealthy (sick) leaves. Leaves were taken from trees in McCreadys Creek, Barnes Creek and Bakers Creek.

Chlorophyll *a* in Water Column

Water samples from Bakers Creek, McCreadys Creek and Barnes Creek were analysed for chlorophyll levels in order to determine the impact of nutrient inputs into the estuaries. Chlorophyll *a* can indicate if there is an excess of nutrients in the water. All three estuaries had levels of chlorophyll *a* exceeding the values in the ANZECC water quality guidelines for tropical Australia for slightly disturbed ecosystems (0.002 mg/L). In each estuary, the highest chlorophyll *a* levels were detected in the upper river sites. The Pioneer River (within Barnes Creek) had the highest level of chlorophyll *a* (0.035 mg/L) of all estuaries at the site PW1 (Figure 42). Once out of Barnes Creek into Bassett Basin and the mouth of the Pioneer River, the chlorophyll *a* levels dropped to below guideline levels. Bakers Creek also had high levels of chlorophyll *a* (0.025mg/L) upstream, however, levels are still above guideline levels at the mouth (Figure 43). Due to unfavourable weather conditions, samples could not be taken directly at the river mouth of Bakers Creek. Levels of chlorophyll *a* within McCreadys Creek did not reach such high levels as detected in the Pioneer River and Bakers Creek, however did still just exceed guidelines at 3 of the upstream sites (Figure 41).

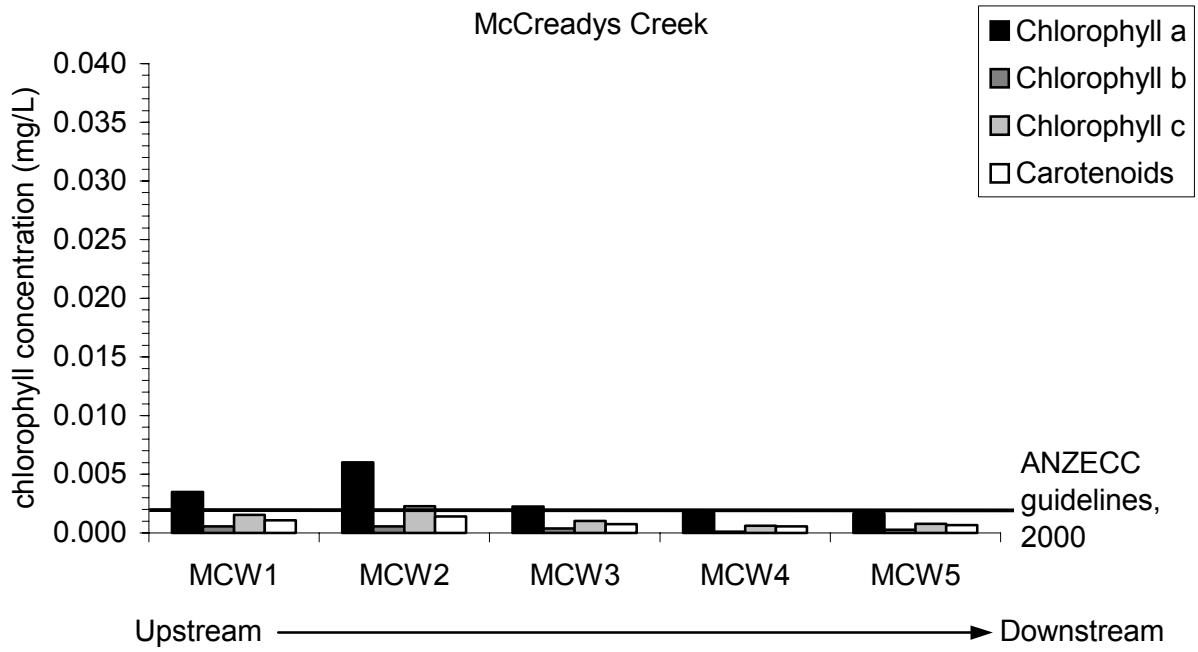

FIGURE 41: Chlorophyll *a*, *b*, *c* and carotenoid concentrations in McCreadys Creek, with ANZECC guideline (2000) levels for chlorophyll *a* for slightly disturbed ecosystems in tropical Australia. Sites are located from upstream to downstream. Refer to Figure 4 for details of locations.

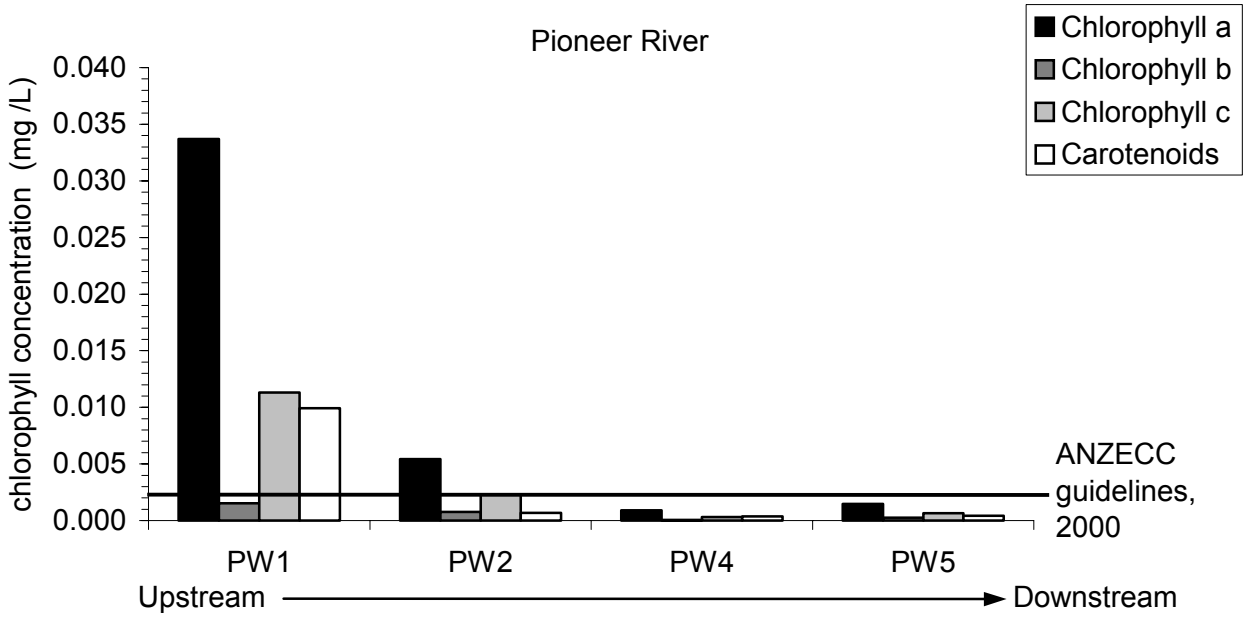

FIGURE 42: Chlorophyll *a*, *b*, *c* and carotenoid concentrations in the Pioneer River, with ANZECC guideline (2000) levels for chlorophyll *a* for slightly disturbed ecosystems in tropical Australia. Sites are located from upstream to downstream. Refer to Figure 5 for details of locations.

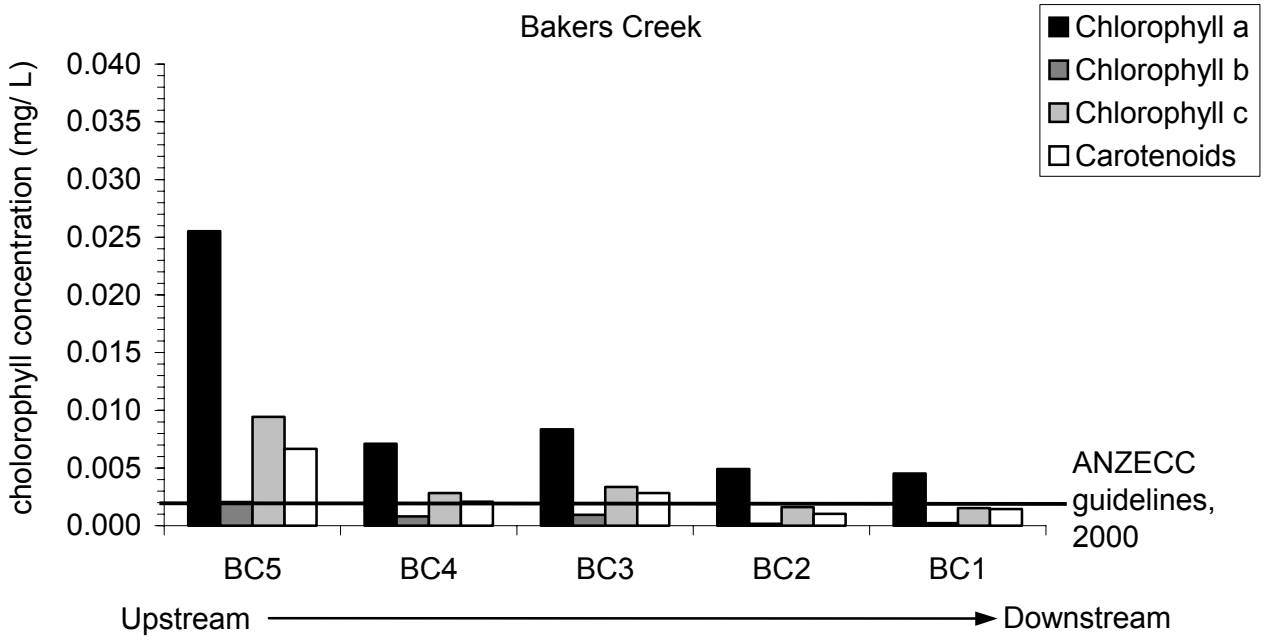

FIGURE 43: Chlorophyll *a*, *b*, *c* and carotenoid concentrations in Bakers Creek, with ANZECC guideline (2000) levels for chlorophyll *a* for slightly disturbed ecosystems in tropical Australia. Sites are located from upstream to downstream. Refer to Figure 6 for details of locations.

Sedimentation

Pneumatophore Height

Distance of *A. marina* pneumatophores from cable root to the sediment surface was significantly higher at BCS1 (Bakers Creek) than any other site (Figure 44). All averaged pneumatophore heights were higher that 5 cm in each plot.

A one way ANOVA was conducted to determine whether there was any difference between sites. There was no significant result for below-ground pneumatophore distances, however there was a significant result for above ground pneumatophore heights. A LSD test was conducted to determine which sites were significantly different. A significant ($p<0.05$) difference between Bakers and Barnes Creeks was found, with Barnes Creek pneumatophores being significantly taller (8.81cm) than those at Bakers Creek (7.01cm). McCreadys Creek pneumatophores (7.825cm) were not significantly different from either Bakers Creek or Barnes Creek.

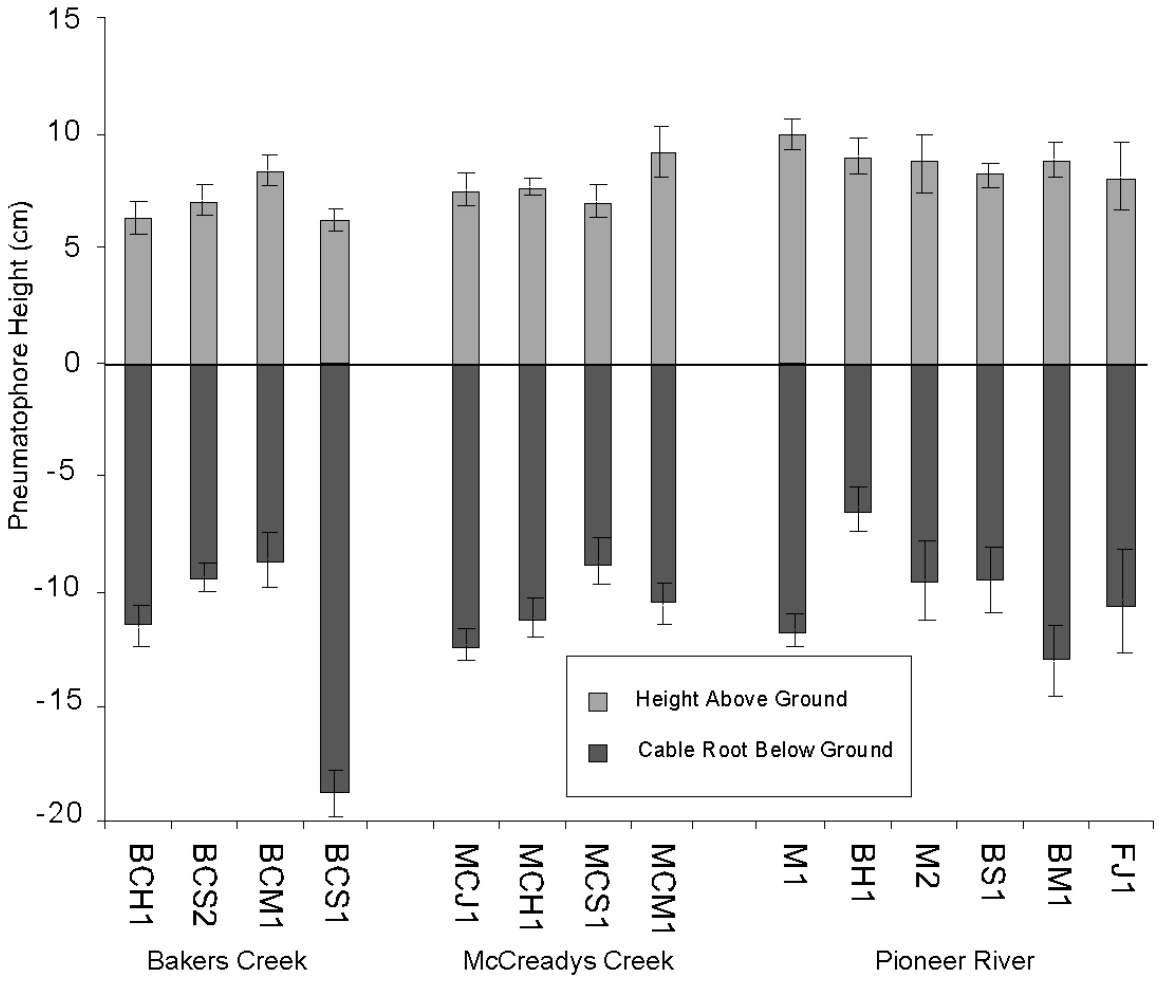

FIGURE 44: Pneumatophore heights above the sediment surface and distance below ground to cable roots of *A. marina* for each of the mangrove plots in the 3 estuaries. For location of mangrove plots, refer to Figures 4, 5 and 6. Error bars are standard error terms for replicated samples.

Herbicides

Herbicides were measured from most plots in the sediment, core water, and leaves within mangrove plots which were chosen as having severe, moderate and no dieback of *A. marina* within the plots. Herbicides screened in this investigation included the substituted ureas and the triazine herbicides, specifically diuron, ametryn, atrazine, fluometuron, hexazinone, prometryn, simazine and tebuthiuron. The results of the analyses are presented in Tables 13, 14 and 15.

Sediment

Detectable amounts of diuron in mangrove sediment were found at all sites within each of the three estuaries (Table 13), with the two highest values both being found in Barnes Creek, within the Pioneer River estuary (sites BM1 and BS1). Sites within Bakers Creek and McCreadys Creek were also found to have comparably high levels of diuron. The highest level detected in the preliminary investigation (Duke *et al.,* 2001) was 4 µg/kg at site M1. Levels of diuron found in this investigation exceeded that value in 7 out of the 11 sediment samples analysed. In comparing values for sites M1 and M2 between the two sampling times, levels in the sediment increased slightly, but taking experimental error into account, they could be considered to be approximately the same concentration. Ametryn was detected in the mangrove sediments at all plots in Barnes Creek and Bakers Creek, however there was none detected in McCreadys Creek. The highest values of ametryn were found in Barnes Creek at the same two sites where the highest level of diuron was also detected. Levels of ametryn in sites M1 and M2 were lower than measured in the preliminary investigation in 2000. Atrazine was found in two plots, within Barnes and Bakers Creeks. As with ametryn, there was no atrazine found in McCreadys Creek mangrove sediments. Levels of ametryn and atrazine were low in comparison to the levels of diuron detected in the sediments.

Leaves

Diuron was detected in the leaves of a sick *A. marina* tree at one site in Bakers Creek (Table 14). No other herbicide was detected in leaves of *A. marina*.

Water

A greater number of herbicides were detected in the water sampling compared to the sediment sampling (Table 15). The herbicides found in the water were simazine, diuron, atrazine, ametryn, hexazinone and tebuthiuron. As with the sediment, diuron was detected the most frequently of all herbicides, and was found all sites, except for site BCH1 at Bakers Creek. The highest levels of diuron in the water of mangrove plots were detected in Barnes Creek in plots BH1 and BS1, which were also the plots with the highest sediment detection of diuron. Samples taken from the river water in the Pioneer River detected diuron both at PW1 (the highest point of Barnes Creek accessible by boat) and at PW5, close to the Pioneer River mouth (Refer to Figure 4 for sites). Surface water sampling from agricultural drains detected quite high levels of herbicides, in particular the cane drain that runs into Fursden Creek, which had a total diuron concentration of 1.15 µg/L.

TABLE 13: Herbicide content of mangrove surface sediment (0-5 cm) in mangrove plots throughout Barnes Creek, McCreadys Creek and Bakers Creek in the Mackay area, with comparisons for plots M1 and M2 from the preliminary investigation (2000). Refer to Figures 4, 5 and 6 for site locations. ND indicates that no herbicides were detected.

Herbicides in Sediment		**Detected Herbicides (µg/kg dw)**					
		Atrazine		**Ametryn**		**Diuron**	
Site Name	**Code**	**2000**	**2002**	**2000**	**2002**	**2000**	**2002**
McCreadys Creek	MCH1	ND	**ND**		**ND**		**1.7**
McCreadys Creek	MCS1	ND	**ND**		**ND**		**6.0**
McCreadys Creek	MCM1	ND	**ND**		**ND**		**1.2**
Barnes Creek	M1	ND	**0.1**	**0.2**	**0.09**	**4**	**5.1**
Barnes Creek	M2	ND	**ND**	**0.1**	**0.09**	**0.8**	**1.0**
Barnes Creek	BH1	ND	**ND**		**0.28**		**7.9**
Barnes Creek	BS1	ND	**ND**		**0.57**		**8.2**
Bakers Creek	BCH1	ND	**ND**		**0.08**		**2.4**
Bakers Creek	BCS2	ND	**ND**		**0.11**		**4.3**
Bakers Creek	BCM1	ND	**ND**		**0.08**		**4.3**
Bakers Creek	BCS1	ND	**0.2**		**0.08**		**6.2**
Detection Limit		0.1	**0.1**	**0.05**	**0.05**	**0.1**	**0.1**

TABLE 14: Diuron concentration (µg/kg) in the leaves of healthy and unhealthy (sick) *A. marina* plants from McCreadys Creek, Barnes Creek and Bakers Creek. Refer to Figures 4, 5 and 6 for site locations. Note: ND indicates that there was no herbicide detected in the leaf.

Herbicides in leaves		**Detected diuron (µg/kg)**	
		***A. marina* health**	
Site	**Code**	**healthy**	**sick**
McCreadys Creek	MCH1	ND	**ND**
McCreadys Creek	MCM1	ND	**ND**
McCreadys Creek	MCS1	ND	**ND**
Barnes Creek	BH1	ND	**ND**
Barnes Creek	BS1	ND	**ND**
Barnes Creek	M1	ND	**ND**
Barnes Creek	M2	ND	**ND**
Bakers Creek	BCH1	ND	**ND**
Bakers Creek	BCM1	ND	**ND**
Bakers Creek	BCS1	ND	**3**
Bakers Creek	BCS2	ND	**ND**
Detection Limit		1	**1**

TABLE 15: Herbicides in water (core water, cane drain and water column sampling) from McCreadys Creek, Pioneer River Estuary (Barnes Creek and Fursden Creek) and Bakers Creek. Refer to Figures 4, 5 and 6 for location of sites. Note: ND indicates that herbicide was not detected in the sample; and, 1000 ng/L = 1 µg/L.

Site Code	Site Location	Detected Pesticides (ng/L)					
Mangrove Plots (Core Water)		**Simazine**	**Diuron**	**Atrazine**	**Ametryn**	**Hexazinone**	**Tebuthiuron**
MCH1	McCreadys Ck	16.42	8.36	ND	ND	3.18	ND
MCS1	McCreadys Ck	ND	7.57	ND	0.69	ND	ND
M1	Barnes Ck	ND	6.49	ND	ND	ND	ND
M2	Barnes Ck	ND	9.14	ND	ND	ND	ND
BH1	Barnes Ck	ND	14.11	ND	1.45	3.67	ND
BS1	Barnes Ck	ND	12.92	ND	2.7	4.95	ND
Fursden Creek	Fursden Creek	2.14	9.24	1.84	0.77	ND	ND
BCH1	Bakers Ck	ND	ND	ND	ND	2.76	ND
BCS2	Bakers Ck	ND	8.98	ND	ND	ND	ND
BCM1	Bakers Ck	ND	3.89	ND	ND	ND	ND
BCS1	Bakers Ck	ND	8.23	ND	ND	3.62	ND
Transects (Core Water)							
MCT1	McCreadys Ck	ND	3.32	ND	ND	ND	16.12
MCT1	McCreadys Ck	ND	4.32	ND	ND	2.42	ND
BT1	Barnes Ck	ND	9.86	ND	0.84	ND	ND
BCT2	Bakers Ck	9.36	8.17	ND	ND	ND	ND
Drain Sampling							
Fursden Drain	Fursden Ck	ND	1151.05	10.23	1.03	5.94	ND
Bakers drain	Bakers Ck	ND	534.31	47.34	2.34	260.79	ND
Water Column Sampling							
PW1	Pioneer R (upstream)	ND	5.24	ND	ND	1.95	ND
PW5	Pioneer R (downstream)	ND	1.07	ND	ND	ND	ND

toxY-PAM

% Average algal inhibition (*Phaeodactylum tricornutum*) using the toxY-PAM was varied even with repetitions from the same sample varying on a large scale. There was a weak relationship between diuron concentration in the mangrove core water and stream water and average % algal inhibition within each sample (r^2=0.3655, p<0.05, n=10). – Figure 45. Research is ongoing for this potentially powerful tool (Bengtson-Nash, PhD. thesis).

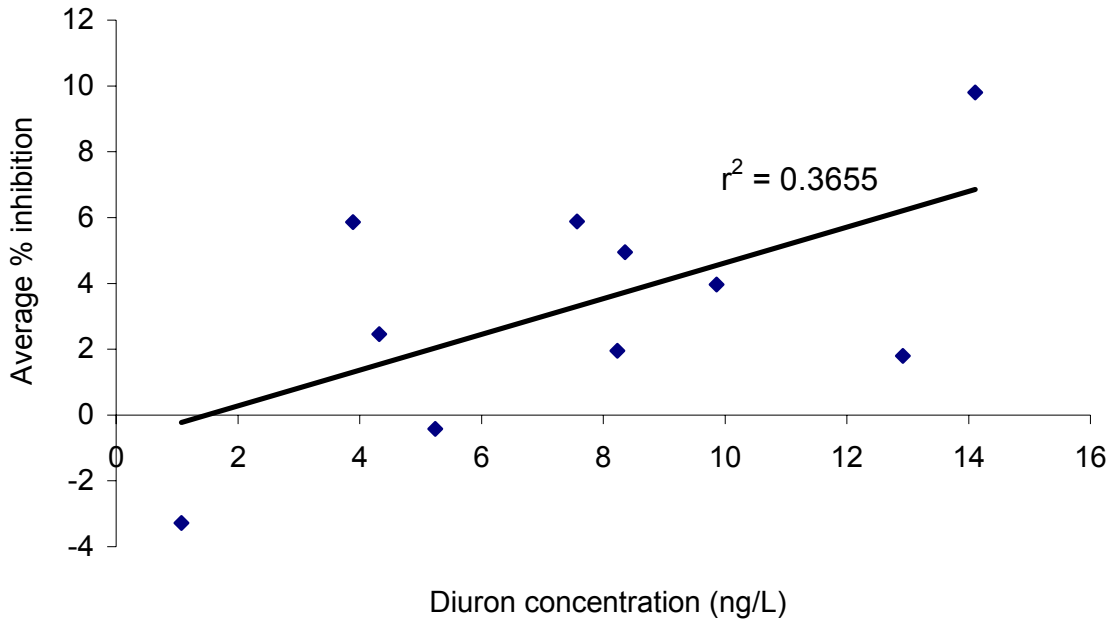

FIGURE 45: Relationship between the average % algal inhibition in the toxY-PAM and the sediment core water and river water diuron concentration (r^2=0.3655, p<0.05, n=10) from samples in the Mackay dieback areas.

Discussion

Conduct of This Investigation

As in the preliminary investigation (Duke *et al.,* 2001), there were three chief aspects of this investigation, including a synthesis of: supportive evidence (including rainfall data, pesticide reports, and so on); the assessment of spatial extent of dieback in the region from aerial surveys; and, the gathering of field observations and samples to compare with aerial survey observations, and to assess forest condition correlated with likely dieback agents in sediments, leaves and water. Two additional aspects have also been essential in answering the many questions raised, and these have been presented in latter sections of this report, namely the planthouse trials, and the comparative rivers study. Each aspect has been integrated in this assessment of factors likely to cause the dieback, or be related to it.

The following discussion provides details considered regarding each of the agents most likely to cause dieback, including: heavy metals, excess nutrients, pneumatophore burial, herbicides and accessory factors.

Extent and Progression of Dieback

Although dieback was occurring in five estuaries, the data on the extent of dieback in the current investigation covers only four estuaries (three of which had dieback) including Eimeo/Bucasia, McCreadys Creek, Pioneer River and Bakers Creek. Concurrent with the observations in the preliminary investigation, the Eimeo/Bucasia region was still unaffected by dieback. The extent of dieback in McCreadys Creek and Bakers Creek was less than in the Pioneer River, with dieback occurring as isolated patches. Comparatively, aerial surveys of mangrove areas in the Pioneer River showed that notable dieback dominated most sections of the estuary from the river mouth to the furthest extent of mangroves upstream (Figure 16). Around 55% of mangrove areas were affected by dieback, which mainly affected one species, *A. marina*. Furthermore, dieback occurred wherever *A. marina* was located, including the entire tidal range from low to high intertidal stands. There were no areas with *A. marina* that were not affected by moderate or severe dieback.

Since *A. marina* alone showed the effects of the agent causing the dieback, it must be concluded that the agent causing this dieback probably occurs throughout all mangrove areas in the estuary, perhaps inhibiting seedling recruitment and affecting forest composition. It is possible these effects also may have been active over a number of years in which case the absence of *A. marina* now cannot be seen as evidence that this species did not normally grow there. The trees may have died earlier because of the agent, and subsequently rotted away.

A future investigation needs to look more closely at mapping the extent of dieback in the past. It appears that dieback might have commenced much earlier than August 1998 contrary to the hypothesis proposed by Kirkwood and Dowling (2002). A series of photographs taken by Mr de Pinto in May 1998 clearly show dieback of *A. marina* in many parts of Bassett Basin, Pioneer River (Figure 47). The great number of dead *A. marina* trees in May is clear evidence that the August 1998 unseasonal flow event was not specifically related to the dieback in question. In addition, a preliminary inspection of aerial photographs of Fursden Creek taken between 1993 and 1997 (complements of Mackay Sugar), show notable loss of *A. marina* trees in this upstream location in Pioneer estuary. Based on such evidence, it appears the onset of dieback might have been as early as the mid 1990's, and it might have occurred in upstream mangrove stands first. There is an urgent need to carefully map the dieback from such aerial photographs and to accurately define the extent in several increments of years between 1990 and 2002.

Field data indicated that the mangrove dieback had worsened between 2000 and 2002. Two plots (M1 and M2) from the preliminary investigation (Duke *et al.,* 2001) were re-surveyed in the current investigation (Figure 46) and in both plots the health of *A. marina* trees had decreased in plots M1 and M2 over the 2-year period, with the appearance of more sick and dead trees in plot M1 and an increase in sick trees in plot M2.

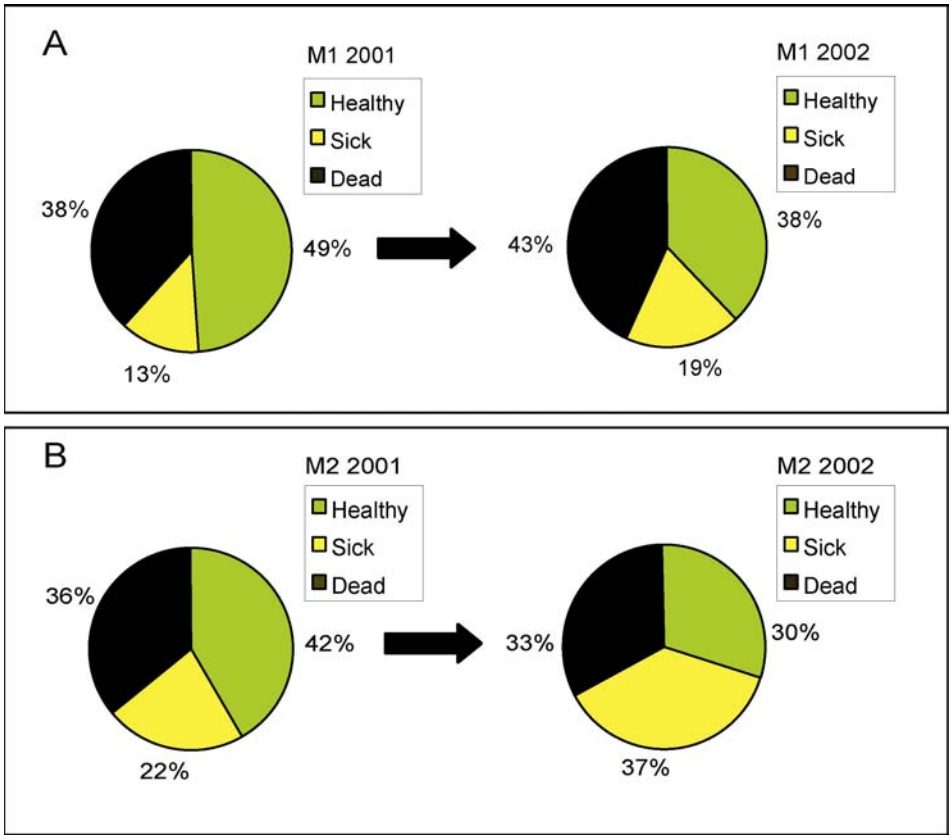

FIGURE 46: Mangrove plots M1 (A) and M2 (B) comparing the health of *A. marina* in 2000 (Duke *et al.,* 2001) and 2002 (current investigation).

FIGURE 47: Mouth of Bassett Creek, draining into the Bassett Basin (Pioneer River Estuary) with freckled patches of sick and dead *A. marina* amongst other live species. Photos by Mr. Serge de Pinto (25 May,1998).

Likely Agents Causing/ Contributing to Dieback

Heavy Metals as a Possible Cause

Sediment and Core Water

The concentrations of heavy metals found in Mackay in the sediment were not higher than average levels, nor did any of the sediment levels exceed ANZECC (2000) guidelines. Furthermore, the levels of heavy metals in Mackay sediments were lower than the levels found in the Johnstone River, in plots where there were healthy stands of *A. marina* (Table 16 and see the Comparative Rivers section). There was no correlation between the level of dieback and the heavy metal concentrations in the sediment or water.

Leaves

Concentrations of heavy metals and elements in the leaves of *A. marina* were not different to the average levels (Table 16), except for Fe, which had a normal average range, but at some sites, had higher concentrations than normally found. In metal polluted environments, plants may display visible toxicity symptoms such as leaf discolouration, chlorosis and necrosis, or may not have visible symptoms at all (Bargagli, 1998). Mangroves are different to most terrestrial plants, in that they are able to accumulate heavy metals to a greater extent before exhibiting signs of stress (Yim and Tam, 1999).

The concentrations of elements in the healthy and unhealthy *A. marina* leaves varied for each element. These elements could be divided into three groups depending on their relative abundance in the *A. marina* leaves as (i) those with more in the healthy leaves than the sick leaves (Cu, Zn and Cl), (ii) those with more in sick leaves than healthy leaves (Ca, Mn and Mg), and (iii) those with similar or varying amounts (K, Na, Cr and Fe).

Where there were more elements in healthy leaves than sick leaves, this might suggest that (i) the dieback was being caused by a deficiency of these elements in the environment. This is highly unlikely however, given that there were healthy trees in the vicinity with higher concentrations of these elements; (2) the agent causing mangrove dieback may have been preventing assimilation of these elements, or (3) a nutrient transfer mechanism may have been taking place in the leaves of the sick *A. marina* trees. Nutrient transfer involves plants withdrawing nutrients from senescing leaves, increasing the availability of these nutrients for other parts of the plant (Wang and Lin, 1999). Although the leaf dropping of these sick trees was not due to senescence (old age) it is possible that a similar mechanism for nutrient conservation was occurring.

Similarly, in leaves with a greater concentration of elements in the sick leaves, it is possible that mangroves may have been actively disposing of unneeded molecules into the sick leaves (Jamale and Joshi, 1976). In a study on senescent leaves of *Sonneratia acida*, *Exoecaria agallocha* and *Lumnitzera racemosa*, (Jamale and Joshi, 1976) accumulation of chlorides, sodium and calcium into the senescent leaves was accompanied by withdrawal of potassium. These were not the same elements detected in the sick trees in Mackay however, and perhaps this was due to the nature of the dieback, as mentioned before, these leaves were not senescent. Also, the dieback agent may have affected mechanisms of transport in and out of the leaves.

TABLE 16: Levels of heavy metals found in average concentrations in sediments and leaves, concentrations found in healthy *A. marina* sites from Johnstone River mangrove sediments and ANZECC (2000) trigger values, compared with the values for the mangroves in the Mackay area from McCreadys Creek, Pioneer River and Bakers Creek. Numbers in brackets indicate arithmetic mean values of samples. A blank entry means that no data was available.

	Sediments (mg/kg)				**Leaves (mg/kg)**		
	Normal sediment	Mackay dieback sites	Johnstone River with Healthy *A. marina*	Trigger Values - ANZECC (2000)	Normal leaves	Mackay healthy *A. marina* leaves	Symptoms
Lead (Pb)		3-13 (7.6)	10-11 (10.3)	50			
Copper (Cu)	10-80 (a)	8-33 (19.4)	33-46 (38.7)	65	2-20 (a)	6-14 (8.75)	Chlorosis, alterations in root growth (a)
Manganese (Mn)	20-3000 (a)	63-250 (125.1)	190-560 (430)		10-100 (a)	19-130 (65.0)	Marginal leaf chlorosis and necrosis, puckering of leaves (a)
Zinc (Zn)		13-51 (31.8)	61-74 (68.6)	200	10-300 (a) 15-800 (b)	9.1-23 (15.0)	Chlorosis, stunted growth (a)
Cadmium (Cd)		<1	<1	1.5			Chlorosis, necrosis, wilting, red-orange leaves, growth reduction (a)
Chromium (Cr)		8-34 (18.9)	69-100 (89.3)	80		0.4-20 (3.9)	
Arsenic (As)		2-11 (6.2)	9-15 (11.3)	20			
Mercury (Hg)		<0.2	<0.2	0.15			
Iron (Fe)		6500-29000 (16827)	37200-48300 (41133)		25-300 (b)	57-1000 (223.8)	
Nickel (Ni)	5-50 (a)	3.6-16 (9.2)	36-56 (46.3)	21			Chlorosis, inhibition of leaf expansion, deformed leaf tissues (a)

(a) – Prasad, 1999
(b) – Raven *et al.,* 1992

Using Heavy Metals to Trace Runoff

Fertilisers may contain trace metals as impure substances, which can result in significant additions to the soil in the long term (Giuffre de Lopez Camelo *et al.,* 1997). As such, it may be possible to give an indication of the impact and source of runoff from terrestrial land because metals are also strongly associated with particulates.

Arsenic (As), Cadmium (Cd), Copper (Cu), Mercury (Hg) and Zinc (Zn) have the most potential impact and enter the environment as a consequence of agricultural activity (Haynes and Johnson, 2000) such as the use of inorganic phosphate fertilisers (Bargagli, 1998). The presence of detectable concentrations of Cd and Hg in recently deposited marine sediments strongly suggests that some agricultural soil is getting to the near-shore environment (Cavanagh *et al.,* 1999). However, when this information was applied to this study, it was determined that the heavy metal 'fingerprints' of the Mackay estuarine sediments collectively differ from those of the upstream canelands in Mackay (Rayment *et al.,* 1997, 1998) – See Table 17.

TABLE 17: Average concentrations of metals (mg/kg dw) found in mangrove sediments in the current investigation in Mackay, compared with concentrations of the same metals in Mackay canelands.

Source	As	Cr	Cu	Ni	Pb	Zn
McCreadys Creek	5.7	14.3	10.7	6.8	5.0	19.3
Barnes Creek	4.8	15.0	20.0	7.6	9.5	34.3
Bakers Creek	8.0	26.3	25.3	12.7	7.8	38.8
Average Mackay Mangroves	6.18	18.9	19.36	9.22	7.63	31.81
Mackay canelends (Rayment *et al.,* 1997, 1998)	3	71	21	17	17	45

However, there are several factors likely to alter the proportion of elements in estuarine mud flats. Four reasons include;

1) Relative dispersal rates of these heavy metals in runoff are unknown. It is likely that these heavy metals will adhere to sediments in different ways and amounts so that the relative ratios between agricultural lands and mangrove/estuarine sediments are not expected to be comparable.

2) The original background levels of these heavy metals in the mangrove sediments are not known. Heavy metals and trace elements come from natural sources such as rock weathering and other geochemical processes involving leaching of continental rocks and sediments (Baskaran *et al.,* 2002).

3) Plants may utilise these heavy metals in disproportionate ways, some are essential elements (Zn, Cu, Ni and Cr), while others are not required by plants, and are toxic at high concentrations (Pb and As) (Raven et al., 1992).

4) Additional metals are added to the environment from discharge of effluent (such as the Mt. Bassett Sewage Treatment Plant, the aquaculture farm and the meatworks facility) and urban stormwater (Haynes and Johnson, 2000), and will therefore interfere with the concentrations present in runoff from agricultural areas.

Nutrients as a Possible Cause

Mangrove Core Water

Sediment nutrients occur in three interrelated forms: dissolved in the sediment porewater/ corewater, adsorbed to the surface of the sediment particles, and fixed within the lattice structure or matrix of the sediment grains. Sediment corewater nutrients are biologically available to organisms within the sediment and can become available to the water column (Dennison and Abal, 1999).

It is obvious that nutrients are a major problem in the Mackay region. Every single sample exceeded the ANZECC (2000) guidelines for total N, total P, ammonia and total dissolved P. The majority of samples also exceeded the guidelines for filtered reactive P.

From the results, it appears that while all three estuaries have significant nutrient problems, Bakers Creek has the biggest nutrient problem of the three estuaries studied, given that trends show the highest mean values for total N (with McCreadys), total dissolved N, ammonia, total P and total dissolved P. Elevated nutrient levels in Bakers Creek could be related to a number of sources including irrigation tailwaters or agricultural runoff, septic tank discharge or stormwater runoff and discharge from sugar mill ponds (Arthington *et al.,* 2001). Both an aquaculture farm and a meatworks facility (Figure 57A and Appendix 14) discharge into the tidal region of the creek, however water samples from a freshwater site (Arthington *et al.,* 2001) above the influence of the meatworks and aquaculture have shown similar trends of nutrients to the rest of the estuary as well as having very low dissolved oxygen levels, which suggests that the vast majority of nutrient input is located upstream.

Compared with Bakers Creek, the Barnes Creek (Pioneer River) sites had less of a problem with some nutrients, with lowest total N, total dissolved N (with McCreadys Creek), ammonia and total P, however problem nutrients for Barnes Creek were N oxides (with McCreadys Creek) and total dissolved P. In the Pioneer River, significantly elevated levels of nitrogen and phosphorus have been recorded in previous sampling events, apparently associated with sewage outflows and urban stormwater runoff (Arthington *et al.,* 2001). The Mount Bassett Wastewater Treatment Plant was built in 1963 (Mackay City Council, pers comm.) and is located adjacent to the Bassett Basin Fish Habitat Area and continuously discharges secondary treated sewage effluent into an unnamed creek within Bassett Basin (McAuliffe, 1998). Recent efforts have been made to reduce the nutrient output from the Sewage Treatment Plant (Appendix 15). NRM (2002) measured total nitrogen (N), total phosphorus (P), oxides of N, and filterable reactive phosphorus at water sampling sites in the Pioneer River catchment during high flow conditions in 2002 and found that all of these nutrients exceeded guideline trigger values (for aquatic ecosystem protection for slightly disturbed systems in northern Australia).

McCreadys Creek had an intermediate nutrient problem. Dissolved oxygen also appeared to be an issue in McCreadys Creek. DO concentration varies with water temperature, salinity, photosynthetic activity and microbial activity, and may vary widely, particularly where there is significant input of nutrients (ANZECC, 2000). At reduced DO concentrations, many toxic compounds become more toxic, including ammonia, zinc, lead, copper (Davis, 1975). Investigation of dissolved oxygen in McCreadys Creek would require a more robust sampling strategy, as the DO concentration may vary widely over a 24 hour period. It would also involve investigating why DO concentrations were low, whether it be from nutrients, runoff of sugar from cane harvesting operations (which induces a very high biological oxygen demand), or any other phenomenon such as water temperature.

The ratio of TN: TP is commonly used to evaluate the nutrient status of a water body (ANZECC, 2000). When the N: P ratio is greater than 16, water is said to be P deficient, and when it is less than 16 it is N deficient. Data from the three Mackay estuaries suggest that these waters are N

deficient, or more appropriately, P enriched. TN: TP can give an indication of potential for nuisance plant growth or algal blooms, however TN: TP will be of little value in turbid Australian systems where high turbidity can limit growth despite the availability of adequate nutrients (ANZECC, 2000).

The association of this nutrient excess with the mangrove dieback are not clear. Excess nutrients alone have never previously been directly linked with mangrove dieback. There is some concern (Rayment, G. pers comm.) that the abundance of ammonia (NH_3) in the area may be associated with the dieback. At high concentrations, ammonia is toxic to aquatic biota, and the toxicity increases with decreasing dissolved oxygen concentrations, which may be important in McCreadys Creek, where dissolved oxygen levels were measured below ANZECC (2000) guideline levels. Ammonium ions (NH_4+) are not easily transported across cell membranes and therefore do not usually pose a major toxicity threat to aquatic organisms. The un-ionised form (NH_3) however easily crosses cell membranes. It usually only becomes prevalent and therefore an environmental issue in alkaline natural waters. By pH 9.0 and over, most of the ammonium-N would be present as un-ionised ammonia. The core waters were not sufficiently alkaline (to pH 8.8) to postulate the presence of sufficient free ammonia to possibly adversely affect the health of mangroves, however the creek waters were.

Ammonia concentrations were not correlated with the dieback. If ammonia was the cause of dieback, it would not explain why mangrove condition was worst at Barnes Creek of all 3 estuaries, when it had the lowest mean ammonia concentration. However, it is possible that there may be synergistic effects of these nutrients with other agents such as diuron that may be detrimental to mangrove health. Given that there is a significant relationship between the amount of ammonia and total nitrogen in the mangrove corewater, and %N in the mangrove leaves (see section below), it seems highly likely that an excess of nutrients may facilitate the uptake of another toxic compound.

In any case, the levels of nutrients in the core water are undesirably high for healthy tropical estuarine ecosystems, and given the already degraded status of these estuaries, particularly the Pioneer River estuary, it is important that steps be taken to reduce the amount of nutrients reaching these waters, whether from point sources (sewage treatment plant, meatworks) or non-point sources (urban runoff, agricultural lands).

Leaves

δ15N - Nitrogen stable isotopes enable detection and delineation of sewage-derived N from other N sources. Atmospheric N exists in two stable isotopic forms, 14N and 15N. The most abundant form is 14N (~99.6%), with 15N comprising a much smaller fraction (~0.4%). The relative proportion of 15N to 14N is referred to as $\delta^{15}N$, measured in ppt (Dennison and Abal, 1999). Sewage-derived nitrogen was present in all estuaries, particularly Barnes Creek, reflecting the impact of the Mt. Bassett sewage treatment plants on mangroves in Bassett Basin. Levels of $\delta^{15}N$ in Barnes Creek in the current investigation were in the same range as detected in the preliminary investigation (Duke *et al.,* 2001).

% Nitrogen – Boto and Wellington (1983) detected significant increases in nitrogen and phosphorus content in *R. stylosa* leaves at a site that had been enriched with ammonium and phosphate for one year, compared with adjacent untreated site. In the current investigation, percentage nitrogen in the leaves of *A. marina* in Mackay correlated with concentrations of Total N and Ammonia in the sediment core water. This shows that uptake of nitrogen is clearly correlated with its presence in the environment and excess nutrients will be reflected in the leaves.

Carbon 13 (δ13C) - Plant tissue $\delta^{13}C$ has been correlated with vegetative growth, climate, atmospheric CO_2 concentrations, nutrient availability, water use efficiency and pollutant stress (McNulty and Swank, 1995). Atmospheric levels of CO_2-carbon contain 12C and 13C at a ratio of approximately 99:1 (Stuiver *et al.,* 1982). When stomata are open, atmospheric CO_2 is transported readily into leaf intercellular spaces, therefore 12C is preferentially fixed compared to 13C

(O'Leary, 1981). When stomata are closed, intercellular CO_2 supply is limited and the 13C/12C fraction ($\delta^{13}C$) of plant material increases, because proportionally more 13C is incorporated into photosynthate (McNulty and Swank, 1995). Leaves from unhealthy trees had a higher 13C/12C ratio than leaves from healthy trees, suggesting that the factor causing the dieback was causing stomatal closure in the unhealthy leaves. Photosynthetic inhibition by herbicides results in an increase in CO_2 content of the sub-stomatal cavity, causing stomatal closure and lower transpiration rates (Chaerle *et al.,* 2003).

Stream Water/Chlorophyll *a*

Chlorophyll *a* occurs in all plants that produce O_2 by photosynthesis and can indicate if there is an excess of nutrients in the water, because all plants, cyanobacteria and algae contain about 1-2% dry weight chlorophyll *a*. Excess of nutrient enrichment and therefore chlorophyll *a* enrichment is associated with a number of problems such as simulation of excessive algal growth and deoxygenation of the water by microbial activity. Measuring only nutrients in a water column does not give an indication if there are any problems with plant growth, as other factors such as turbidity may exclude growth of plants in the water. Measuring chlorophyll *a* can give an actual indication if there is a problem, and appropriate action can be taken for the management of this problem (ANZECC, 2000).

Levels of chlorophyll *a* in the water column in Mackay were high in each estuary and exceeded the ANZECC guidelines in each estuary. The peak chlorophyll levels were detected in the Pioneer River, followed by Bakers Creek and McCreadys Creek, in spite of the fact that the highest average nutrient concentrations were detected in the mangrove corewater in Bakers Creek.

The lower amount of flushing in Barnes Creek/Bassett Basin may explain why the chlorophyll *a* levels at sites PW1 and PW2 were over the ANZECC limit, but were below the limit in the main section of the Pioneer River. As suggested in the preliminary investigation (Duke *et al.,* 2001), a detailed study of hydrology and water quality needs to be conducted within Bassett Basin to identify and improve the specific issues related to the flushing of the area. Bakers Creek, on the other had had chlorophyll *a* levels over the ANZECC guidelines at every site, but presumably has better flushing and tidal exchange than Bassett Basin does. Although the Bakers Creek estuary has significantly higher values of nutrients than other Queensland estuaries (Baffle, Boyne, Burrum, Elliot and Kolan), there have been no reports of algal blooms (Chl *a*) or eutrophication (pH and oxygen levels) (Arthington *et al.,* 2001), however this is not to say that these high nutrient levels do not pose a problem for the health of the estuary.

The high concentrations of chlorophyll *a* may be influencing the clarity of the water. Water quality measurements of the three estuaries in this study show high turbidity levels (as measured by secchi depth). Light can penetrate to a depth of approximately 1.7 times the secchi depth; therefore lower readings indicate turbid or coloured water. Levels of suspended solids are highly variable, and depend on a range of conditions such as particle size, water turbulence, available wave energy, water depth, seabed characteristics and seasonal fluctuations in sources (Commonwealth of Australia, 1996). Suspended material can include suspended silt or soil particles, phytoplankton and zooplankton and organic matter and may be derived from natural sources such as natural runoff, water turbulence from storms, wave action and phytoplankton blooms. It can also be the result of human activities, such as runoff from agricultural fields, wash from construction sites, shoreline erosion or waste effluent (Commonwealth of Australia, 1996).

Pneumatophore Burial as a Possible Cause

Land clearing in the catchments of the Mackay region, especially in the Pioneer River, has left little remnant vegetation (which is now in bad condition) (Hill and Hunt, 1992) resulting in unnaturally high loads of sediment and nutrient loads in the River, which may be affecting the water quality (see previous section).

Long-term studies on sedimentation in the Mackay region will involve the measurements of sediment accretion/erosion from stakes established in the area, but in the short-term nature of this report, data on pneumatophore burial or erosion could be determined through measuring the pneumatophore heights.

Pneumatophore Height

One aspect of this report was to investigate the possibility that the widespread mangrove dieback might be caused by pneumatophore burial and consequently death by the blockage of respiratory surfaces. The mangroves *Avicennia* and *Sonneratia* are examples of genera that possess 'pneumatophores' also known as peg roots or pencil roots. Both these mangroves occur in Mackay region but there was no indication of *Sonneratia* dieback. Pneumatophores are erect lateral branches of the shallow radiating cable roots (Jenik, 1978; Tomlinson, 1986). Pneumatophores of *A. marina* range in height from a few cm to a reported maximum of 35cm (Snedaker *et al.,* 1981).

Mangroves with pneumatophores are often considered to be susceptible to sedimentation, however other species are also vulnerable, and there are reports of *Rhizophora mangle*, *Avicennia germinans*, *Laguncularia racemosa* and *Conocarpus erectus* death due to sediment deposition (Craighead and Gilbert, 1962). Therefore, the higher stilt-root architecture of *Rhizophora* spp. (for example) does not necessarily mean that they can tolerate deeper root burial (Ellison, 1998). Furthermore, in Mackay, there was no evidence that the widespread dieback of *A. marina* in the Pioneer River or adjacent estuaries, was caused by pneumatophore burial, although a number of isolated incidents indicated this form of dieback does take place in the region (eg. plots MCS1 and BCS1). However, this sediment-related dieback included *R. stylosa* and *C. australis*, as well as *A. marina* (See Figure 57B for a picture of sediment-related *R. stylosa* dieback).

Mangroves with pneumatophores (*Avicennia* and *Sonneratia*) and knee roots (eg. *Xylocarpus mekongensis*) also have the ability to extend their breathing roots in response to sedimentation (Hutchings and Saenger, 1987 p. 28). Mangrove species with other structures (eg. buttress roots, and stilt roots) may also be able to adapt to gradual burial (Ellison, 1998). Data from the current study on pneumatophore height show that the height of the above-ground components of the pneumatophores are fairly consistent throughout each plot, suggesting that the *A. marina* trees that are alive do have exposed breathing components. One site, BCS1 had a significantly longer length of pneumatophore to the cable root suggesting that some sedimentation has taken place in the past, however *A. marina* has grown fast enough to keep up with the sedimentation rates.

Herbicides as a Possible Cause

Detection of Herbicides in Sediment and Corewater

A number of herbicides were detected in the Mackay estuaries, specifically diuron, ametryn, atrazine, simazine, hexazinone and tebuthiuron. All of the herbicides detected were urea or triazine herbicides, which act by inhibiting electron transport in Photosystem-II of the electron transport chain in photosynthesis (Percival and Baker, 1991). These Photosystem-II inhibiting herbicides are all root-absorbed, and some are also absorbed by foliage (Gerst, 1999).

The herbicide concentrations in the sediment and water in the sampling locations in Mackay varied quite significantly on a small spatial scale. There were a greater number of herbicides detected in the water samples (6) than in the sediment (3), due to the fact that detection of herbicides was measured in nanograms in the water and in micrograms in the sediment. Pesticide concentrations are often 2-3 orders of magnitude higher in sediments than in the associated water (Wauchope, 1978). The highest level of herbicides in the mangrove plots found in both water and sediment were found in Barnes Creek (Pioneer River estuary), then Bakers Creek, and then McCreadys Creek.

Appendix 14 provides an overview on the properties of the herbicides found in Mackay, and their respective ANZECC guidelines for water quality. All herbicides detected in this investigation have been described as “leachers” (Betiz *et al.,* 1994) as they are generally highly mobile, travel rapidly to the water table, and have moderate to high half lives, allowing them to persist in the sub-surface environment (Baskaran *et al.,* 2002). As a consequence, many of these herbicides in particular diuron, atrazine and ametryn, have been frequently detected in other sampling events, such as in mangrove sediments (Duke *et al.,* 2001), irrigation drains (Müller *et al.,* 2000), groundwater (Baskaran *et al.,* 2002) and runoff (NRM, 2002). Samples taken during a three-day rainfall event (13-15 February, 2002) by NRM (2002) detected ametryn, atrazine, hexazinone, 2,4-D and diuron within the Dumbleton Weir in the Pioneer River. Diuron was detected at 8.50µg/L and the estimated load of diuron for the whole event was 470kg, much higher than the estimated loads for all other pesticides during the event (NRM, 2002).

The herbicides in the Mackay region were most likely to be derived from terrestrial agricultural runoff, as contribution by other potential sources (eg. antifouling paint on boat hulls and roadside weed control) were considered to be minimal, especially in the Pioneer River (See Duke *et al.,* 2001 p. 58). The sugarcane industry is the highest user of the herbicides detected in the mangrove sediments in Mackay and the herbicides atrazine and diuron in particular are among the most popular in use in the central sugarcane region of Queensland - see Appendix 18 It is important to note that tebuthiuron, a herbicide found in the mangrove core water at one location in McCreadys Creek, can only be legally used in Eucalypt woodlands [broadleaf (silverleaf) ironbark] in Queensland, so at least some of the sediment probably comes from this source. Diuron was the main herbicide in question after the preliminary investigation into the mangrove dieback (Duke *et al.,* 2001) and more recently has been under scrutiny, with a chemical review of diuron being currently undertaken by the National Registration Authority.

Diuron in Mangrove Sediments and Core Water

As with the preliminary investigation (Duke *et al.,* 2001), the herbicide diuron was found in most locations at the highest concentrations of all the herbicides detected. Diuron concentrations in mangrove sediments and core water in Mackay were relatively high ranging up to 6-8 µg/kg for sediments and 8-14 ng/L for core water in the 3 estuaries sampled (Table 15, Figures 4, 5 and 6). It was notable that maximal values were detected in Barnes Creek, Pioneer River estuary, while levels in the other two estuaries were around half that level (see Figure 48).

Currently, there are no ANZECC (2000) guideline values, or there is insufficient guideline data for the herbicides levels detected for marine estuarine sediments or water. Other guidelines do exist

for diuron in sediments, such as in the Netherlands, where the maximum permissible concentration for diuron in sediment is 9 µg/kg, and in soil it is 8 µg/kg (Crommentuijn *et al.,* 2000). This deficiency of data for Australian ecosystems needs to be addressed urgently.

The concentrations of diuron in the sediment and core water were associated with each other (Figure 48). The amount of herbicide in water and sediment depends on a number of factors including the properties of the herbicide and the sediment. Some pesticides are highly soluble in water but because of their ionic properties, they will bind tightly to the soil particles and pose minimal risk for groundwater contamination. The organic matter in soil is one of the main soil constituents that adsorbs pesticides. Less soluble pesticides like atrazine are less tightly bound to soil particles and can move in both sub-surface drainage and in surface runoff (Hargreaves *et al.,* 1999). The soil-water partition coefficient (Koc) is an index for pesticide mobility and for a given amount of pesticide applied, the smaller the Koc value, the greater the concentration of a pesticide in solution (Rao *et al.,* 1998). Pesticides with small Koc values are more likely to be leached compared to those with large Koc values (Rao *et al.,* 1998). Reported values for pesticide half-life and partition coefficient vary widely in the literature. A summary of the properties of herbicides detected in Mackay, from the SCS/ARS/CES database for environmental decision-making is presented in Table 18.

TABLE 18: Half-life and soil partition coefficients (Koc) and movement rating of herbicides found in mangrove sediments and pore water in the mangrove dieback areas in Mackay (Wauchope *et al.,* 1992)

	Hexazinone	Tebuthiuron	Atrazine	Simazine	Ametryn	Diuron
Half life (days)	90	360	60	60	60	90
Koc	54	80	100	130	300	480
Pesticide movement rating	Very high	Very High	High	High	Moderate	Moderate

Based on this information, the following formula can be used to convert water herbicide concentration values to sediment concentration values (also see Haynes *et al.,* 2000a)

X µg/kg sediment = (X µg/L x partition coefficient of the herbicide in water x % organic matter) /100

This formula did not apply with the concentrations of herbicides in water and sediment with respect to the organic matter in Mackay (Table 19), as converted values were incorrect by significant amounts compared with actual measured values. It is suggested that the reliability of the above formula be reviewed.

TABLE 19: Diuron concentrations in water and sediment, and % organic matter in sediment, for 3 adjacent estuaries in the Mackay region, McCreadys Creek, Pioneer River, and Bakers Creek, sampled in May 2002. Numbers in brackets refer to the range of data.

	Diuron in water (ng/L)	Diuron in sediment (µg/kg)	% Organic matter in sediment
McCreadys Creek	5.8 (3.3-8.4)	3.0 (1.2-6.0)	9.0 (7.9-9.9)
Barnes Creek	10.5 (6.5-14.1)	5.6 (1.0-8.2)	7.1 (3.5-14.6)
Bakers Creek	5.9 (0-9.0)	4.3 (2.4-6.2)	18.8 (6.3-23.4)

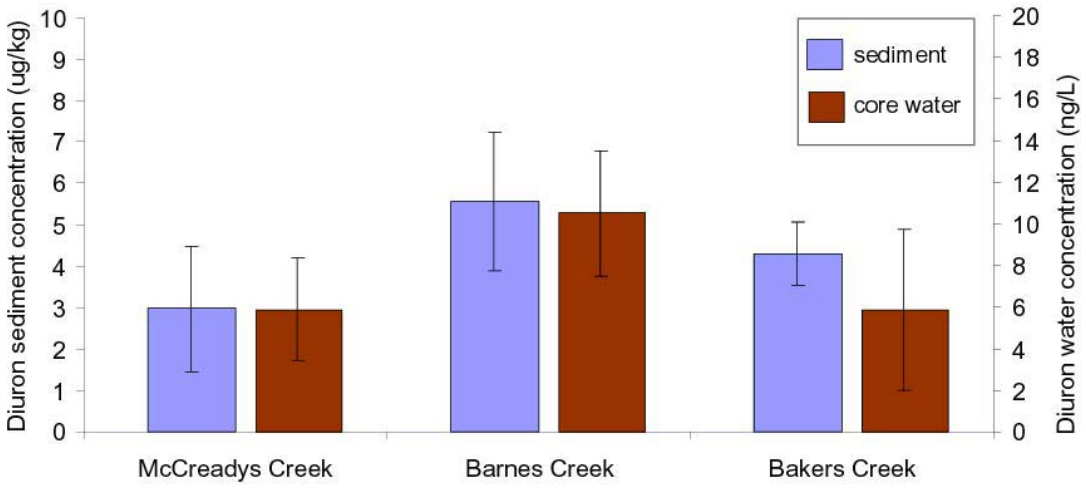

FIGURE 48: Average diuron concentration from the study sites in McCreadys Creek, Barnes Creek and Bakers Creek, comparing concentrations in the sediment (µg/kg) and the mangrove plot core water (ng/L).

Diuron Persistence - The diuron concentrations in sediment in the plots M1 and M2 remained effectively the same from the sampling time in 2000 to the sampling time in 2002, suggesting that either the herbicides did not degrade in the sediments between the two sampling periods, or that there was a constant supply of herbicides to the sites (Figure 49). A similar pattern was seen for ametryn at the same sites (Figure 50). In order to be confident of the breakdown patterns of these herbicides in mangrove sediments, periodic monitoring or studies on degradation rates for mangrove sediments need to be undertaken.

Normally, the dissipation rates (DT50's or half-lives) of modern herbicides are quite rapid, even for compounds considered to be relatively persistent. The half-life may vary depending on soil properties including soil microbial populations, moisture, temperatures and pH. Non-persistent pesticides have half-life of 30 days or less, moderately persistent pesticides have a half-life of 30 to 99 days and persistent herbicides have a half-life greater than 100 days (Rao *et al.,* 1998). Microbial degradation is considered to be the primary mechanism for the degradation of diuron in soil (Tixier *et al.,* 2000) as it is not affected by photolysis and is not volatile from soil (Hamilton and Haydon, 1996). Because of anoxic conditions, sediment bound-herbicides are able to persist for longer periods of time than those in the water (Voulvoulis *et al.,* 2002). A study by Hargreaves *et al*. (1999) however found that in one study site, diuron was available at a significant concentration for offsite movement for extended periods of time after application (nearly 200 days) in red ferrosol soil. This was because it was incorporated within the soil matrix sufficiently to protect it from some of the normal chemical and microbial breakdown processes (Simpson *et al.,* 2001b). If herbicides are not broken down, or broken down slowly in the mangrove sediment, there would be a permanent presence of herbicides in contact with mangrove roots.

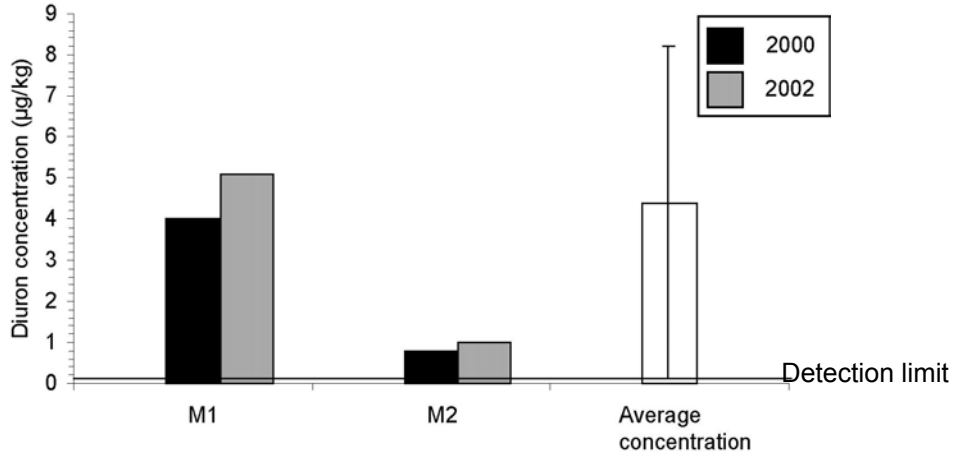

FIGURE 49: Comparison in diuron concentrations between site M1 and M2 over two sampling periods (2000 and 2002). Average diuron concentration and the range of diuron found in these two sites are provided for reference. The detection limit for diuron is as indicated (0.1µg/kg).

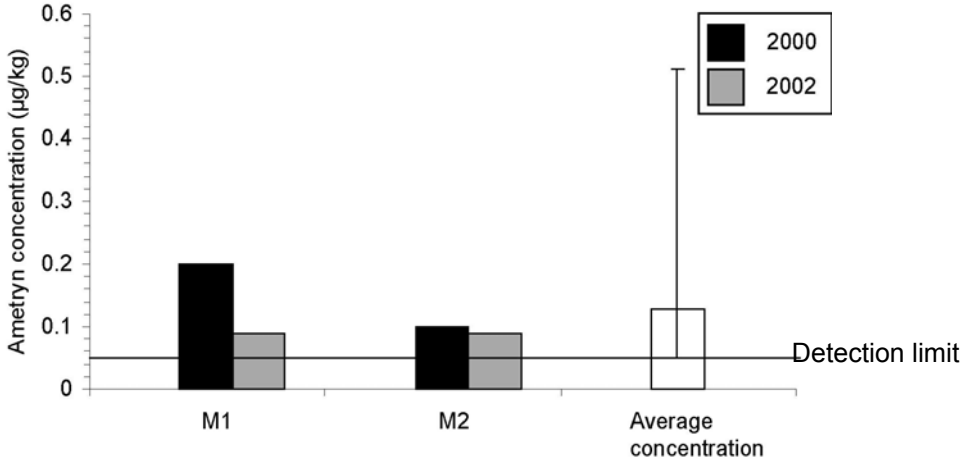

FIGURE 50: Comparison in ametryn concentrations between site M1 and M2 over two sampling periods (2000 and 2002). Average ametryn concentration and the range of ametryn found in these two sites are provided for reference. The detection limit for diuron is as indicated (0.05 µg/kg).

Presence of Diuron and Mangrove Condition

Canopy Dieback Observed in Aerial Surveys - Aerial surveys of the Mackay region provided estimates of mangrove health. The percentage of healthy *A. marina* within each estuary was significantly ($p<0.01$) correlated with the presence of diuron in the mangrove sediments, however the concentration of diuron ranged greatly throughout each estuary, and therefore this relationship was not significant (Figure 51). The two samples from 2000 were not included in the correlation because the areas of healthy trees were only roughly estimated.

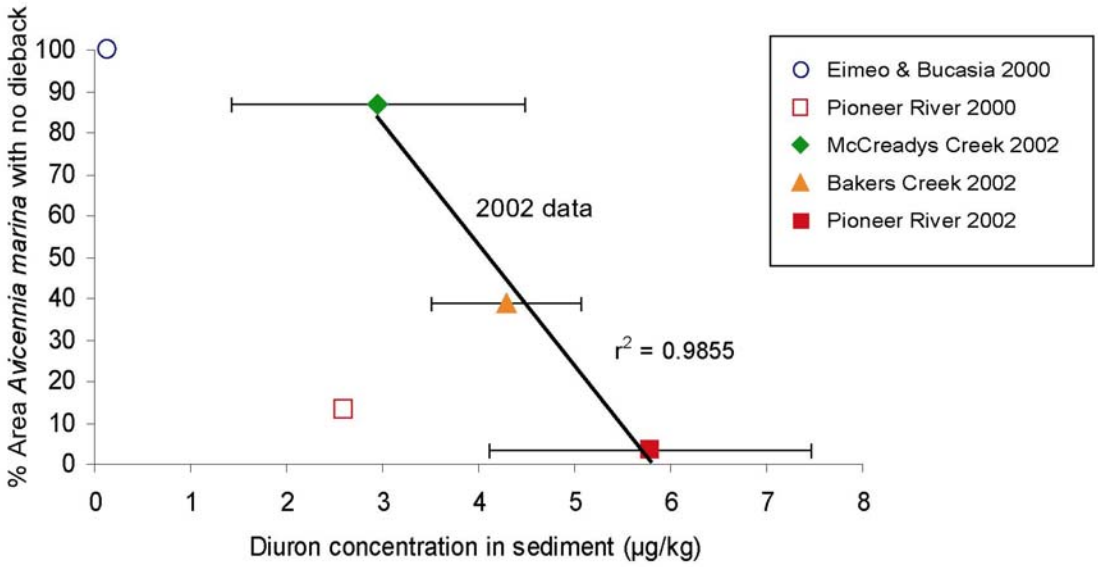

FIGURE 51: Relationship ($r^2=0.9855$, n=3, $p<0.01$) between the average concentration of diuron in the mangrove sediments in the Mackay region and the % area of healthy *A. marina* trees, as quantified by the mapping exercise.

Tree Condition in Plots – In the ground surveys, the majority of plots were classified as severe and all plots within McCreadys, Bakers and Barnes Creek had notable dieback. Mangrove dieback was generally species-specific for *A. marina*, however dieback of other species was observed in some locations. In the Barnes Creek area, especially in the plots BS1 and BM1, leaves of other species were found to be yellowed and dropping off. It is uncertain whether this sickness was related to the *A. marina* dieback, but these plots had quite high herbicide levels both in the sediment and core water. At high herbicide levels, it may be just a matter of time before other species are also affected.

Dieback of *C. australis* was observed in McCreadys Creek area, in and around site MCS1. Aerial photographs of the area confirm the *C. australis* dieback, which is most likely to be caused by a previous sedimentation event. In the same area however, there were healthy *A. marina*, showing that even in areas where sedimentation kills the vast majority of another species, *A. marina* can still survive. It is unknown where the source of sediment in McCreadys Creek originated but this would need to be investigated further. Another suspected sedimentation site was BCS1 within Bakers Creek, where a large proportion of *C. australis* trees in the plot were dead and sick. This site was located immediately downstream from the meatworks and an aquaculture facility. It was also situated close to a bund wall, separating cane farms from the mangroves. Any one of these factors may have caused this patch of localised dieback in this area.

There was a significant ($p<0.02$) negative correlation between the concentration of diuron in the mangrove sediment and the health of *A. marina* trees in the all of the mangrove plots in McCreadys Creek, Bakers Creek and Barnes Creek combined (Figure 52). This correlation existed also for individual estuaries, in Barnes Creek ($p<0.02$) and Bakers Creek ($p<0.05$). No trend existed for the McCreadys Creek *A. marina* trees however. The reason for this is unknown, but could be due to complicating factors such as sedimentation. At approximately the same diuron

concentration in the sediment, there were nearly 20% more healthy trees in Barnes Creek then in Bakers Creek. The slopes of the lines may be a reflection on different conditions within each estuary such as the amount of organic matter in the sediment, which might influence how much herbicide is available to the plants for uptake.

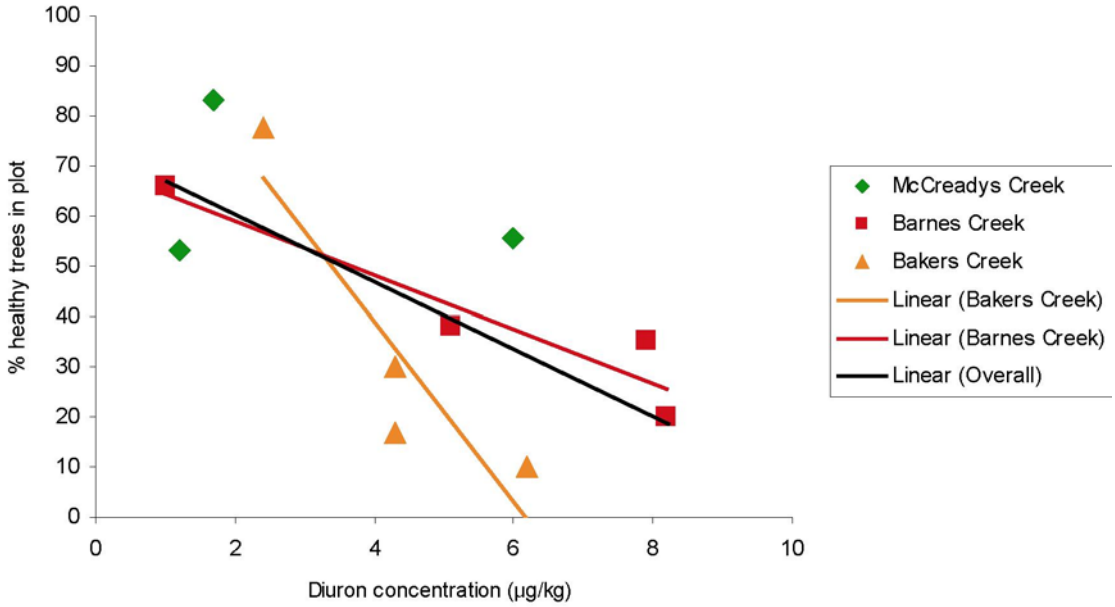

FIGURE 52: Relationship between healthy *A. marina* trees in the plot and diuron concentration in the mangrove sediment (0-5 cm depth) for Barnes Creek (r^2=0.892 p<0.02, n=4) Bakers Creek (r^2=0.8179, p<0.05, n=4) McCreadys Creek (no relationship) and the overall relationship (r^2=0.4803, p<0.02, n=11).

Photosynthetic Activity of Trees - The ratio of F_v/F_m is correlated with the maximum potential yield quantum yield of net photosynthesis of intact leaves, and typically falls in the range of 0.75 to 0.85 (Bolhar-Nordenkampf, 1989). In Mackay, the F_v/F_m ratio was lowest in the *A. marina* trees from Barnes Creek, which was the area where most of the dieback was occurring, and where the highest concentrations of herbicides were found. Levels were below 0.75 for three out of the four plots in Barnes Creek. Although not significantly different, the *A. marina* in areas of moderate dieback appeared to have higher F_v/F_m readings. This is likely to be due to the fact that there were more healthy trees in these plots.

Barnes Creek (in particular, plot BM1) was the only area where species other than *A. marina* showed lower PAM readings, specifically *A. corniculatum* and *B. gymnorrhiza*. There was no herbicide data collected from this plot, however perhaps there is some significance in the fact that Barnes Creek had the highest average diuron concentration of all three estuaries.

Leaf Chlorophyll of Trees - As was expected, lower concentrations of chlorophyll *a* were found in the unhealthy mangroves than in the healthy mangroves. There was a significant correlation between leaf chlorophyll *a* concentration and diuron concentration in each estuary, except for McCreadys Creek (Figure 53). The slope of each line was different for each estuary and this may be because of different estuary conditions, such as sediment type, or nutrient availability.

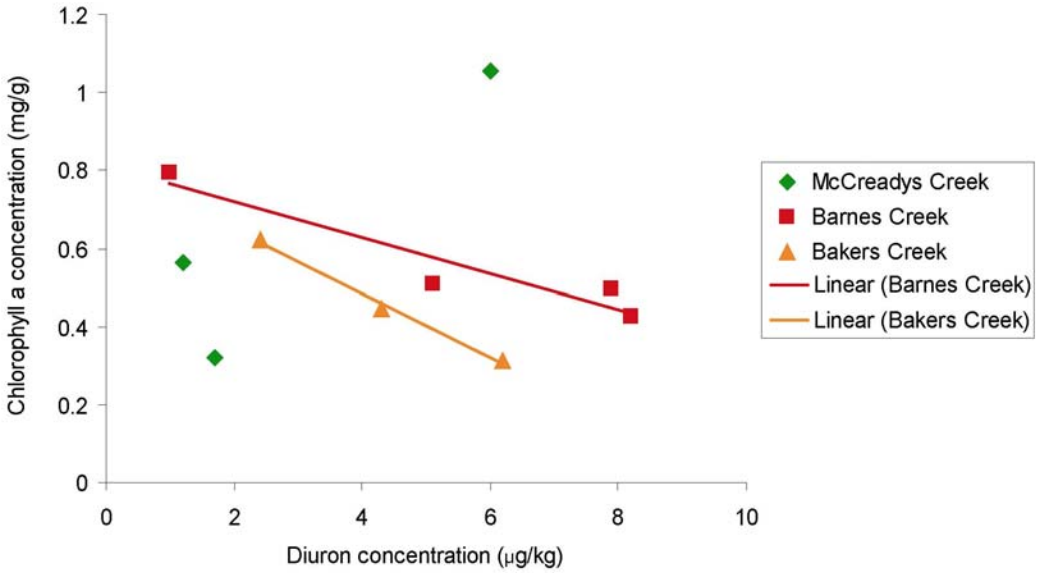

FIGURE 53: Relationship between average chlorophyll *a* concentration (from healthy and sick leaves) in *A. marina* leaves and total herbicide concentration in the sediments for Barnes Creek (r^2=0.8995, p<0.02, n=4) Bakers Creek (r^2=0.9915, p<0.005, n=3) and McCreadys Creek (no relationship).

Seedling Recruitment and Health - There was no clear relationship between herbicide concentration and seedling recruitment, however seedling recruitment does not depend on contaminants in the sediment alone, but also on presence or absence of adult trees supplying the propagules, shading and proximity to the water's edge. In general, Eimeo had the greatest density of seedlings in the plot, however only one plot was surveyed in Eimeo, which did not provide sufficient replication to compare with other areas. As with the trees, there were also plots where other seedling species were sick along with *A. marina* seedlings, in particular, plot BS1 within Barnes Creek, where the highest herbicide levels were found. There was a significant correlation between the percentage of healthy *A. marina* seedlings in Barnes Creek (p<0.05), Bakers Creek (p<0.05) and McCreadys Creek (p<0.005) and the total herbicide concentration in the sediment (Figure 54). There was also an overall significance level of p<0.05 for all three estuaries combined. Differences in the slopes of the lines may be attributed to factors such as the % organic matter in the sediments and nutrient availability to the plot.

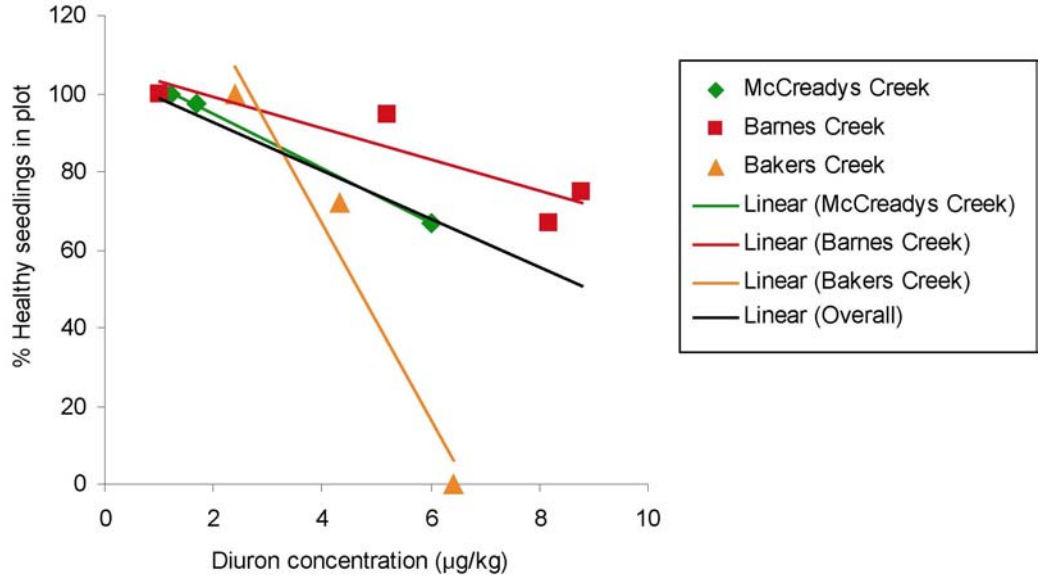

FIGURE 54: Relationship between herbicide concentration in the sediment and % healthy *A. marina* seedlings in each plot in Barnes Creek (r^2=0.8069, n=4, p<0.05), Bakers Creek (r^2=0.9524, n=3, p<0.05), McCreadys Creek (r^2 = 0.9995, n=3, p<0.005) and overall correlation (r2=0.3352, n=11, p<0.05). Healthy seedlings included seedlings with insect attack, as sick seedlings were counted as those with yellow leaves.

Accessory Factors Influencing Mangrove Dieback

Possibility For Increased Herbicides

Increased Rainfall and Flooding

Analysis of rainfall records in the Mackay area since 1870 confirmed that the recent period of more frequent flooding (Appendix 21) and increased runoff coincided with above average rainfall (Appendix 20). This increase in rainfall and intensity of floods resulted in an increased level of erosion and transport and deposition of sediments within rivers (Gourlay and Hacker, 1986), which was most likely to be accompanied by an increase of chemical and nutrient runoff. Above average peaks in rainfall/runoff events in 1995 may also coincide with the start of the dieback (Duke *et al.,* 2001 and start of this discussion), however, dieback was definitely occurring in May 1998 (Figure 47).

Increase in Agricultural Areas

The amount of cane in the district under a cultivated regime has increased dramatically over the last number of years (Table 20), and the propensity for the movement of sediments, nutrients and herbicides has also increased.

TABLE 20: Planted cane and total cane in Mackay between 1997 and 2000 (Trevor Wilcox).

Year	Planted Cane (ha)	Total Cane (ha)
1997	14036	77779
1998	12624	72728
1999	9843 (lot of cane left as standover cane from previous year as paddocks were too wet to harvest)	80371
2000	13408	83362

Additional Likely Synergistic Effects With Herbicides

The presence of herbicides alone may not be solely responsible for the widespread, severe nature of the dieback. It is likely that herbicides act as stress agents, and along with a combination of other factors, the plants are terminally stressed.

Combination of Herbicides

Herbicides are commonly found in combination with other herbicides in the field (Haynes *et al.,* 2000a; Baskaran *et al.,* 2002; NRM, 2002; Current Investigation). Studies using algal bioassays have shown that a combination of herbicides and fungicides can cause a synergistic toxic response in algae (Fernandez-Alba *et al.,* 2002). It is therefore important to consider the combined toxic effects of multiple herbicides in the environment, because mixtures of these chemicals can exert a greater negative impact on an organism than the individual components of the mixture (Fernandez-Alba *et al.,* 2002).

Furthermore, the breakdown products of a herbicide may be just as important or more important than the effects of the initial compound. A study by Tixier *et al*. (2000) on fungal degradation of diuron found that the mono- and didemethylated metabolites of diuron were more toxic to fungi than diuron alone.

Temperature

The temperature in Mackay increased almost 1^{o}C between the years 1960 to 2001 (Appendix 20). The toxicity of herbicides has been found to increase in plants with an increase in temperature (Hatzios and Penner, 1982). However, the influence of temperature increase on herbicide phytotoxicity is dependent upon the particular herbicide, the specific plant and the susceptibility of the plant to the herbicide (Hatzios and Penner, 1982). The adsorption of diuron in soil also increases as the temperature increases (Hamilton and Haydon, 1996).

Nutrients and Herbicide Uptake

Nutrients derived from sewage discharge have been shown to have a beneficial effect on growth and productivity of the mangrove ecosystem (Clough *et al.,* 1983), however if plants are growing faster, then there is the possibility the mangroves are taking up chemicals such as herbicides at a greater rate with the nutrients. In general, increased levels of N, P + K have been shown to result in increased phytotoxicity of specific herbicides (Hatzios and Penner, 1982). Furthermore, the addition of ammonium sulfate, ammonium nitrate, or urea plus ammonium nitrate (UAN) in fertilizers has also shown to increase the effectiveness of post emergent applications (Gerst, 1999). This is particularly important in areas such as in the Bassett Basin and Bakers Creek, where nutrient inputs are high (also where mangrove dieback is most severe).

Other Plant Functions

Mangroves are always associated with cyanobacteria of diverse species. Toledo *et al*. (1995) found that in closed system experiments, N_2 fixation gradually increased when plants of *Avicennia germinans* were inoculated with the filamentous cyanobacterium, *Microcoleus sp*.. Inoculated plantlets were also greener and slightly larger than non-inoculated plants. Herbicides may also affect the health of these associated cyanobacteria, therefore affecting nitrogen availability for the plants. There is no information available on this matter, and further research is needed.

Secondary Consequences of Mangrove Dieback

Sedimentation, Erosion and Slumping

Death of *A. marina* trees has caused, and will continue to cause the sediment from around the mangrove roots to become mobilised, as there is a reduced root structure to bind the sediment together (see Figure 56C). This is also demonstrated in photographs of an erosion gully in Barnes Creek, compared between the years 2000 and 2002 (Figure 56 A and B). The gully has become wider, and loss of sediment around the roots has caused instability of the trees, which have subsequently fallen into the gully. Major gully erosion can also be seen in Figure 56 F.

The mobilisation of sediment has lead to a mass movement of mangrove sediment from the upper intertidal areas to the lower intertidal areas, causing erosion and exposure of roots in the upper zones and burial of mangroves in the lower section. This is likely to have exacerbated the dieback especially in the lower intertidal zones. Apart from exerting further stress on already stressed plants, eroded sediments tend to have a greater proportion of fine particles such as clay and organic matter than the parent soil, which consequently have 1-5 times more sorbed pesticides (Kookana *et al.,* 1998). The implications of this sediment movement are potentially huge, as the scree slopes, which are deposited into the creeks and channels (Figure 56D) will be easily

mobilised in the next large rainfall/runoff event, causing problems for adjacent seagrass beds and coral ecosystems by limiting light and smothering the biota. Figure 55 illustrates this concept.

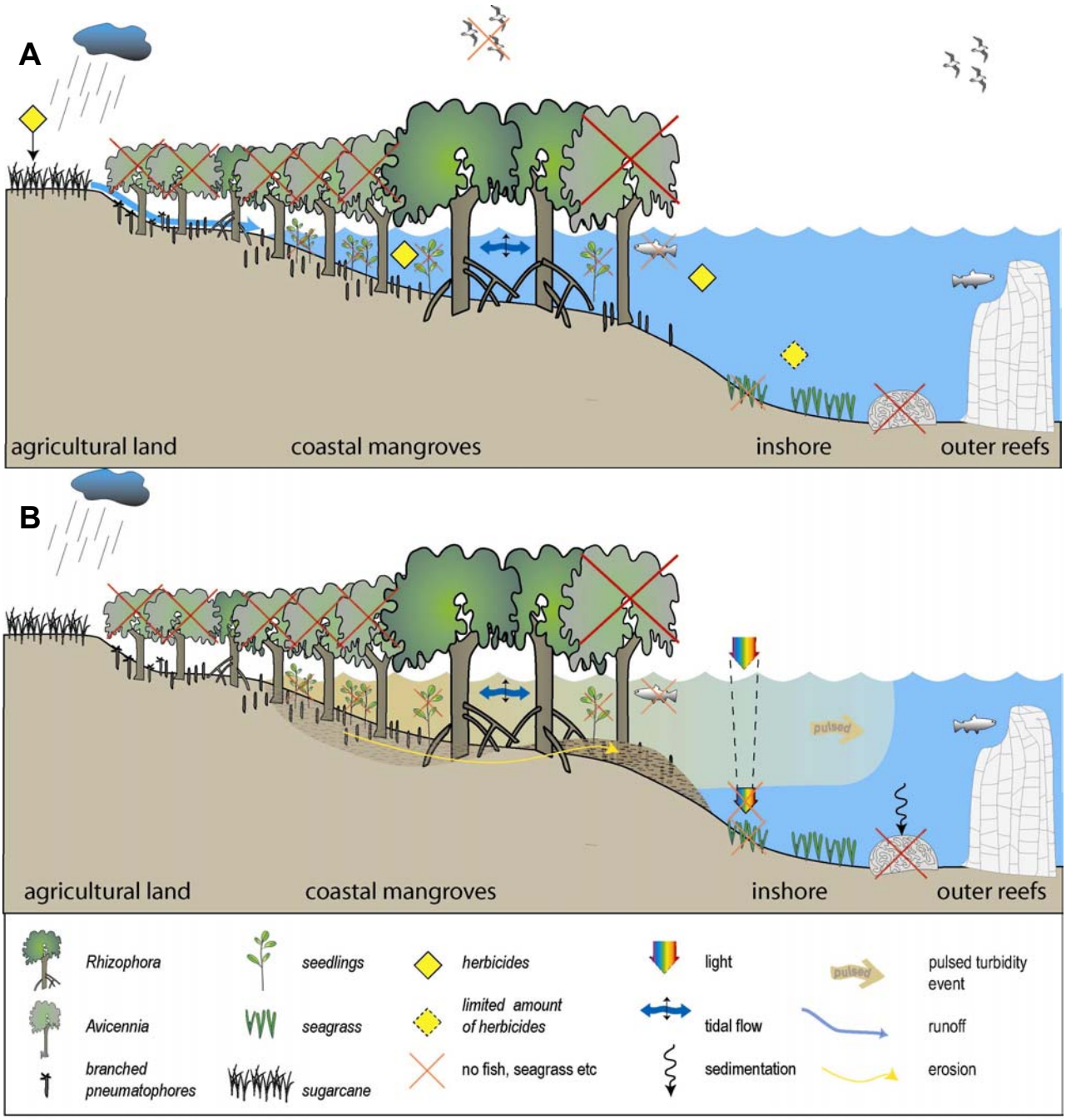

FIGURE 55: (A) Herbicide movement into the marine environment and the effects of herbicides on the biota. (B) Sediment shifting in the mangrove zone, with erosion evident in higher tidal parts where there are dead trees, and with deposition in lower tidal parts along creek margins.

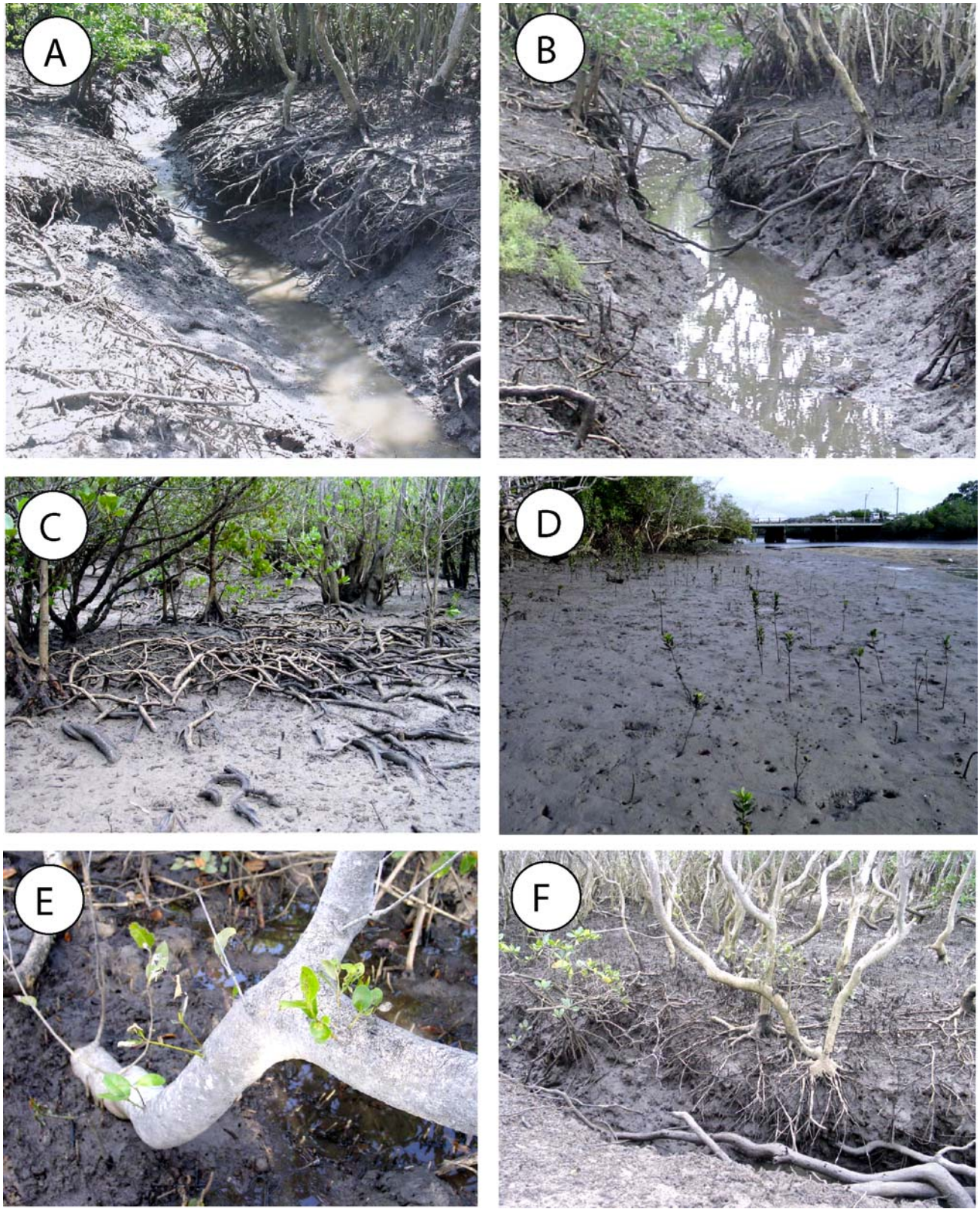

FIGURE 56: (A) An erosion gully in Barnes Creek in 2000 (B) The same erosion gully in 2002 where the gully has widened and trees have fallen over. Salt marsh is moving into light gaps created by the dieback. (C) Major sheet erosion in the upper intertidal areas showing the exposed roots of *A. corniculatum* (D) A scree slope in Barnes Creek with newly established seedlings (E) Resprouting from epicormic buds in *A. marina* (F) Severe gully erosion in Barnes Creek mangroves.

Pneumatophore Branching

During the field studies for this report, there were observations of anomalous pneumatophores, mainly forked, throughout a few sites in the dieback areas in all estuaries. A high frequency of abnormal pneumatophores was observed within a drain runoff area in Bakers Creek (See Figure 57 C and D). These pneumatophores in Bakers Creek were unlike any seen at other sites, as they had branching at the tips into flower-shaped arrangements. The appearance of abnormal-looking pneumatophores is believed to be a response to a persistent sublethal stress in the rhizosphere (the soil that immediately surrounds plant roots), associated death of pneumatophores and an inability to regenerate new pneumatophores in the toxic substrate (Snedaker, 1981). Under these unfavourable conditions, mangroves branch and twist their remaining pneumatophores in order to increase the area available for oxygen uptake, thereby increasing their likelihood of survival (Snedaker, 1981; Mandura, 1997).

Abnormal pneumatophores in mangroves have been observed in other places in the world, in areas where there highly anoxic substrates, impoundment, oil pollution (Böer, 1993), gradual burial of pneumatophores by sedimentation (Hutchings and Saenger, 1987) and sewage discharge (Mandura, 1997). In the current study, the abnormal pneumatophores in the Bakers Creek drain area could be a response to a selection of factors, including ponded water, nutrients derived from cane farms, and herbicides, in particular hexazinone and diuron, which were found at relatively high concentrations. The main route for offsite transport of non-ionic and organic pesticides is largely through attachment to soil particles, (Kookana *et al.,* 1998), and generally the highest risk period for off-site runoff or leaching of a pesticide is immediately after application, when the concentration applied is the highest (Simpson *et al.,* 2001b). In the current investigation, however, high levels of herbicides (especially the herbicides diuron and hexazinone) were detected in clear water draining from a cane farm after an episode of light rainfall, suggesting that these herbicides do not always become immobile when applied to agricultural land and are not always carried off field attached to sediment particles.

Occurrence of abnormal pneumatophores in *A. marina* has not been previously associated with herbicides. The pneumatophores of *A. marina* posses a thin green photosynthetic layer just under the surface of the pneumatophore (Ichiro *et al.,* 1995). The chloroplasts in this photosynthetic layer have been found to be extremely rich in grana stacks as compared with those in the leaf (Ichiro *et al.,* 1995). In addition, the photosynthetic CO_2 fixation activity of the chloroplasts in pneumatophore was more than ten times as high as that in the leaf under lighting condition at the PPFD of 210 mμ-mol m^{-2} s^{-1} (Ichiro *et al.,* 1995). Herbicides may cause inhibition of electron transport in the chlorophyll-enriched areas of the pneumatophore. Given that in other cases, death of pneumatophores causes branching, the same processes could be happening in the Bakers Creek pneumatophores, especially as the pneumatophores are at the sediment/ water level, where the herbicides tend to arrive in runoff. The pathway for intake of herbicides that might affect pneumatophores could be the root tips or the thin respiratory surfaces of the pneumatophores. These observations need to be investigated further since it identifies an additional peculiar feature of *A. marina* that might explain its unusual vulnerability in this dieback.

Epicormic Sprouting

Epicormic sprouting was frequently observed in areas of dieback on *A. marina* trees. Some *A. marina* trees in plot M2 had re-sprouting epicormic buds (Figure 56 E). *A. marina* is a species that can regenerate from epicormic shooting if the apical meristems are damaged (Wadsworth, 1959; Tsuda and Ajima, 1999). It is possible that *A. marina* trees might be able to recover from dieback in some circumstances, however this needs to be investigated further.

Abundance of Benthic Microalgae (BMA)

Photosynthetic pigments are useful indicators of algal biomass. The initial reason for taking sediment cores for BMA was to determine whether there was any correlation between the concentration of herbicides in the sediment, and abundance of BMA. Results show that there was no correlation between these two factors.

Some bacteria and microalgae are equally as sensitive as susceptible higher plants to herbicides (Nystrom *et al.,* 1999), however, as a result of physiological adaptation of microalgae, some species, within limits, can survive in polluted environments (Lopez-Rodas *et al.,* 2001). High interspecies variation in sensitivity to herbicides has been demonstrated for both freshwater and marine microalgae (Kallqvist and Ramstand, 1994; Nystrom *et al.,* 1999).

The most important factor in surviving most environmental changes is the genetic variability in the natural population (Lewontin, 1974; Mettler *et al.,* 1988). Resistance to diuron is due to point mutations at several specific sites in the psbA chloroplast gene (Erickson *et al.,* 1989; Przibilla *et al.,* 1991; Alfonso *et al.,* 1996; Andronis *et al.,* 1998). The rates of these spontaneous mutations vary widely across species and from gene to gene within the same species (Klug and Cummings, 1997). In experimental manipulations with microalgae, it was shown when the culture was treated with a contaminant, the cell density reduced after a few days due to destruction of sensitive cells (Lopez-Rodas *et al.,* 2001). However, when this same culture was incubated for a few more days, the culture increased in density again, due to growth of an algal variant which mutated and became resistant to the action of the contaminant (Lopez-Rodas *et al.,* 2001). Because microalgal populations have high cell numbers and considerable growth rates, the frequency of spontaneous mutations that have been observed in these populations seem to be enough to ensure their survival in contaminated environments (Lopez-Rodas *et al.,* 2001).

Simenstad (1996) conducted an intensive, short-term (119 day) field experiment to determine the potential effects of herbicide control of smooth cordgrass, *Spartina alterniflora* Loisel. on mudflat benthic communities. A mixture of glyphosate (Rodeo; 4.7 L/ha) and an associated surfactant, alkylarylpolyoxyethylene (AAPOE, X-77 Spreader; 1 L/ha) was applied aerially to three mudflat sites with invasive *Spartina alterniflora*. The benthic communities on the mudflat did not display any population trends that would indicate either short- or long-term responses to the herbicide and surfactant applications in the duration of the experiment.

In some locations, it is likely that populations of microalgae will being exposed to these chemicals for the first time, which can be lethal to wild-type strains of microalgae (Lopez-Rodas *et al.,* 2001). It is likely that the BMA in Mackay have been exposed to frequent applications of low concentrations of herbicides in runoff for a number of years, and therefore the current herbicide contamination levels do not affect the present communities. Nevertheless, these resistant mutants have been shown to have diminished growth rates and saturation density and as a consequence, the important role of these BMA (primary production, microalgal biomass) will be severely diminished (Lopez-Rodas *et al.,* 2001).

There appears to be a positive association between the severity of dieback in the plot and BMA abundance, which may be related to the amount of sunlight penetrating to the mangrove forest floor. There would also be a number of other factors interacting with BMA abundance including freshwater availability, nutrient supply and substrate type, including % organic matter (Underwood, 1997).

Mackay Estuaries – Dieback Conclusions

This report has carefully investigated and weighed up a number of agents likely to have caused the species-specific dieback in Mackay region, including heavy metals, nutrients, sediments, and herbicides. Based on careful consideration and analysis of each potential dieback agent, it appears that from all the factors investigated that herbicides, particularly the herbicide diuron was the most likely factor in the dieback of *A. marina* in the Mackay region. The correlations shown in Figures 52-54 indicate with >95% certainty that diuron concentrations were directly and negatively correlated with mangrove health over all estuaries (Figure 52 and 54) and over the 3 separate estuaries (Figure 53). Other factors appear only to have synergistic effects in either enhancing or reducing the severity of dieback.

It is imperative to continue regular monitoring of the condition of mangroves and the presence of herbicides in mangrove sediments, but it is essential and urgent to reduce the levels of herbicides found in mangrove sediments in Mackay region. At risk, are not only the mangrove habitat and associated benefits, but also the adjacent marine habitats including seagrass beds and coral reefs.

The following chapters address specific questions still remaining. These included:

Planthouse herbicide trials –

- Were mangroves affected by herbicides? If so, was *A. marina* more sensitive than other species commonly found in Mackay region?
- Were salt excreting species more sensitive than salt excluding species?
- Was diuron worse than other herbicides found in mangrove sediments?

Comparative rivers study -

- Were mangroves affected by herbicides in other rivers?
- Had species-specific dieback only occurred in the Mackay region?
- Was *A. marina* found in other river systems where herbicide levels were as high as those found in Mackay region?
- Why was the dieback only occurring around Mackay?

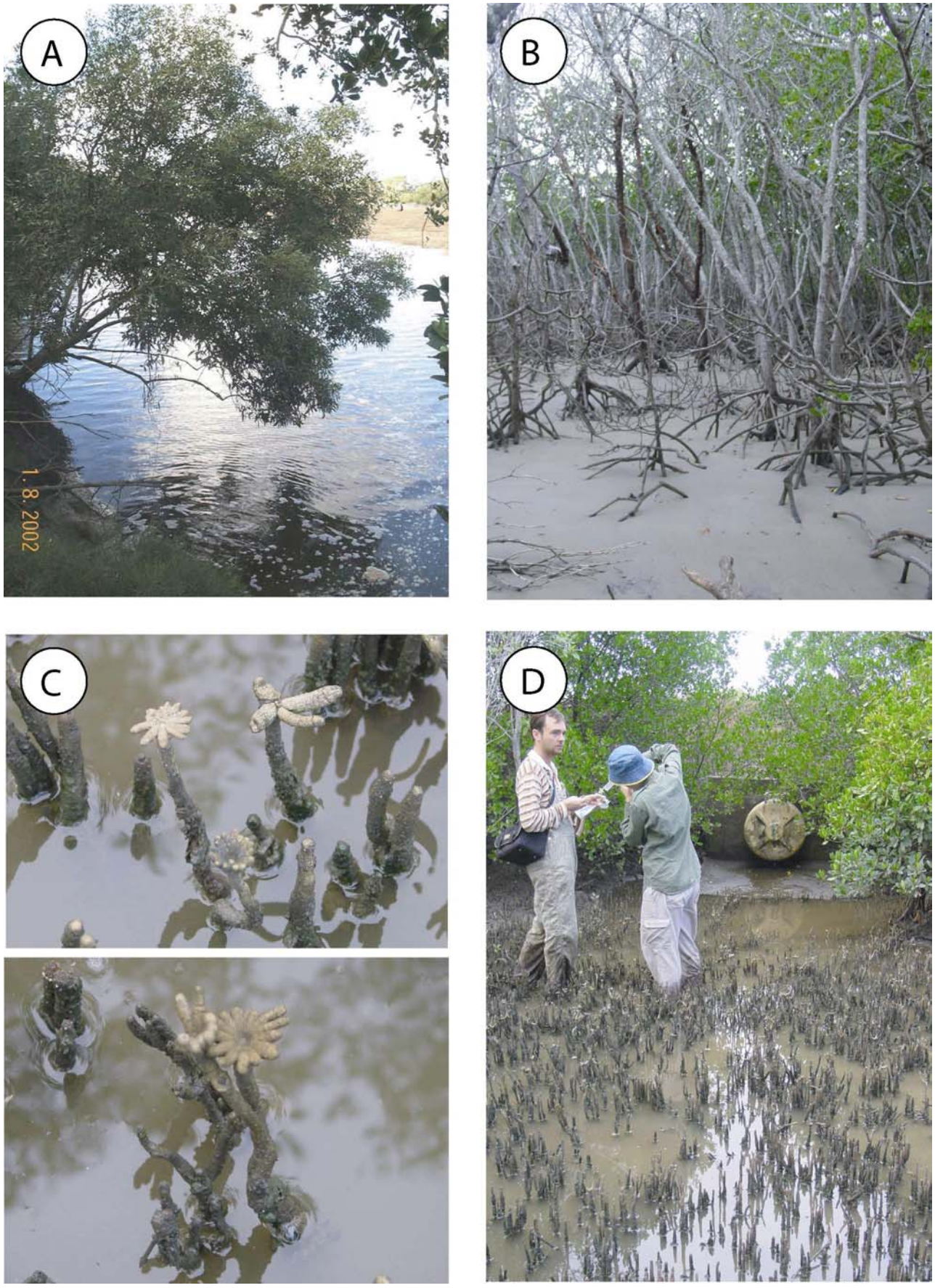

FIGURE 57: (A) Healthy *A. marina* next to Bakers Creek meatworks output. Note bubbly water (Photo Judith Wake) (B) Bakers Creek, with sedimentation affecting *R. stylosa* (C) Anomalous pneumatophores of *A. marina* in drain at Bakers Creek (D) Drain water sampling in Bakers Creek.

Preliminary Toxicology Trials in the Planthouse

Effects of Photosystem II-Inhibiting Herbicides on Four Mangrove Species

Introduction

A specific recommendation of the preliminary investigation into the species-specific dieback in Mackay (Duke *et al.,* 2001) was to "Investigate the physiological response of different mangrove plant species to the presence of toxic substances, particularly for the implicated herbicides, in water and sediment, in controlled tidal nursery experiments".

In excess of 200 different chemicals, with a vast range of properties and applications, are currently classified as herbicides or Plant Growth Regulators (PGR's) (Percival and Baker, 1991). The most widely used commercially available herbicides in agriculture worldwide are the PSII-inhibiting herbicides (Percival and Baker, 1991; DeFelice, 2000).

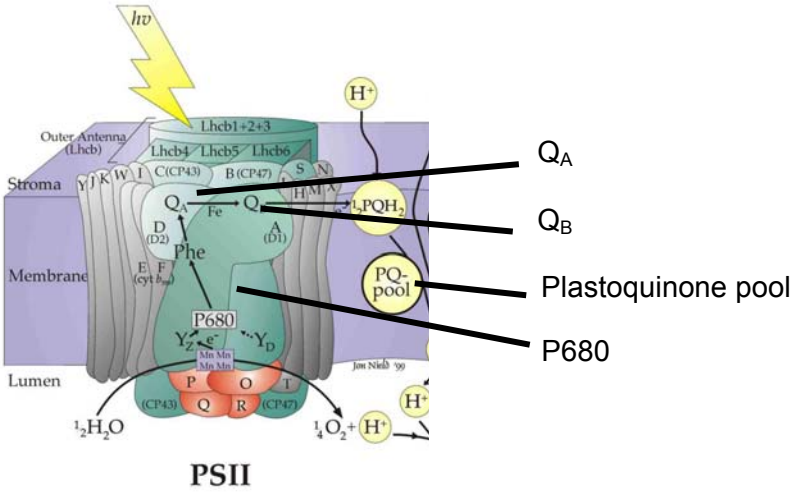

FIGURE 58: Schematic diagram of the Photosystem II complex, situated within the plant thylakoids. Source: Wolfson Laboratories (2002).

Photosystem II (PSII) is a pigment-protein membrane complex (Figure 58), located in the chloroplast thylakoid membrane, and serves to oxidise water on one side of the membrane while reducing platiquinone on the other (Percival and Baker, 1991). In the photosynthetic electron transport chain, electrons are transported from P680 via phaeophytin to the first stable electron acceptor, a plastoquinone (Q_A) (Cobb, 1992). At the reducing side of PSII, Q_A- then transfers its electron to another plastoquinone bound to the Q_B site, located on the D1 protein (Cobb, 1992). Q_B delivers pairs of electrons to the mobile plastoquinone pool (Cobb, 1992).

The primary target of PSII-inhibiting herbicides is the D1 protein. PSII herbicides bind to the Q_B site, inhibiting binding of plastoquinone to the site, so preventing electron flow from Q_A- (inhibiting Q_A- oxidation) (Percival and Baker, 1991). While Q_A is bound tightly to the D2 protein, Q_B is not firmly bound to D1, so herbicides compete successfully with Q_B for this site, occupying space on the D1 protein instead (Cobb, 1992).

Inhibition of electron transfer by PSII herbicides causes a rapid response of chlorophyll fluorescence (Percival and Baker, 1991). Chlorophyll fluorescence measurements provide a

sensitive and early indicator of damage to the photosynthetic apparatus (Schreiber *et al.,* 1994). This can be measured using the saturation pulse method with a specialist instrument called PAM (Pulse Amplitude Modulated Fluorometer) (Walz, Germany). Previous studies have used PAM to determine levels of stress to marine angiosperms (Haynes *et al.,* 2000b, Ralph and Burchett, 1998). PAM fluorometry involves dark adaptation of a plant sample, upon which the minimal fluorescence level, F_o is measured (Schreiber *et al.,* 1994). The F_o level represents the minimal fluorescence associated with maximally oxidised PSII reaction centres of non-energised chloroplast membranes (i.e. the PSII reaction centres are open) (Percival and Baker, 1991). If PSII reaction centres are damaged, as with PSII herbicides, or if transfer of excitation energy from the antenna to the reaction centres is impeded, an increase in F_o will occur (Bolhar-Nordenkampf, 1989). Maximum fluorescence (F_m) is reached when the primary electron acceptor, Q_A is fully reduced and maximal closure of reaction centres to excitation energy occurs (Bolhar-Nordenkampf, 1989). The difference between F_m and F_o is the variable component of F (F_v). The ratio of F_v/F_m is highly correlated with the quantum yield of net photosynthesis of intact leaves, and typically falls in the range of 0.75-0.85 (Bolhar-Nordenkampf, 1989). A decline in F_v/F_m is an indication of photoinhibitory damage caused by light when plants are stressed (Bolhar-Nordenkampf, 1989).

Mangrove physiology may play an important role in determining the species-specific nature of the dieback in Mackay, as species with different physiologies may be capable of translocating different levels of toxins. Mangroves can be divided into two main groups according to their salt regulating characteristics; salt excretors (such as *A. marina, Acanthus, A. corniculatum* and *Aegialitis)* and salt excluders (*R. stylosa, C. australis* and *Bruguiera*) (Clough *et al.,* 1982). All mangroves exclude the majority of salt from the transpiration stream (Ball and Passioura, 1995). Most mangroves possess a barrier to ion transport in the roots (endodermis), at which the salt is filtered out and water is taken up (Scholander *et al.,* 1962). This exclusion of salt is a passive process of membranes in the root, as no inhibition by poisons or chilling has been observed (Scholander, 1968). In the endodermis of plants, channels through which apoplastic movement of water and ions normally occur are blocked by suberin (and lignin in some species), forming a hydrophobic wall called a casparian strip. It is assumed that ions and water cannot move across the band, thereby hindering the apoplastic movement of substances from the cortex to the vascular tissues (Peterson, 1988). Studies on the mechanisms of ion movement in mangroves suggest that mangrove roots possess two casparian bands (Kramer and Preston, 1978; Lawton *et al.,* 1981). Depending on the physiology of the species, rate of ion uptake can vary quite significantly. Differences in the development of primary vascular tissue and in the size of the root cap in *Bruguiera gymnorrhiza* and *A. marina* may explain the differences in the ion uptake in the two species (Lawton *et al.,* 1981). These studies have shown that a casparian strip is present in the walls of the endodermis in the roots of both *A. marina* and *Bruguiera gymnorrhiza* at a distance of 5 mm from the distal end of the root cap – See Figure 59. Differences in the development of this casparian strip may influence ion movement into the stele and hence into the shoots. In *Bruguiera gymnorrhiza*, the casparian strip is immediately adjacent to the proximal end of the root cap, whereas in *A. marina*, there is a 3mm gap between them (Lawton *et al.,* 1981). The inability of *A. marina* roots to prevent salt influx to the extent in which a salt-excluding species might, results in part from this structural gap between the fully developed endodermis and the proximal end of the root tip.

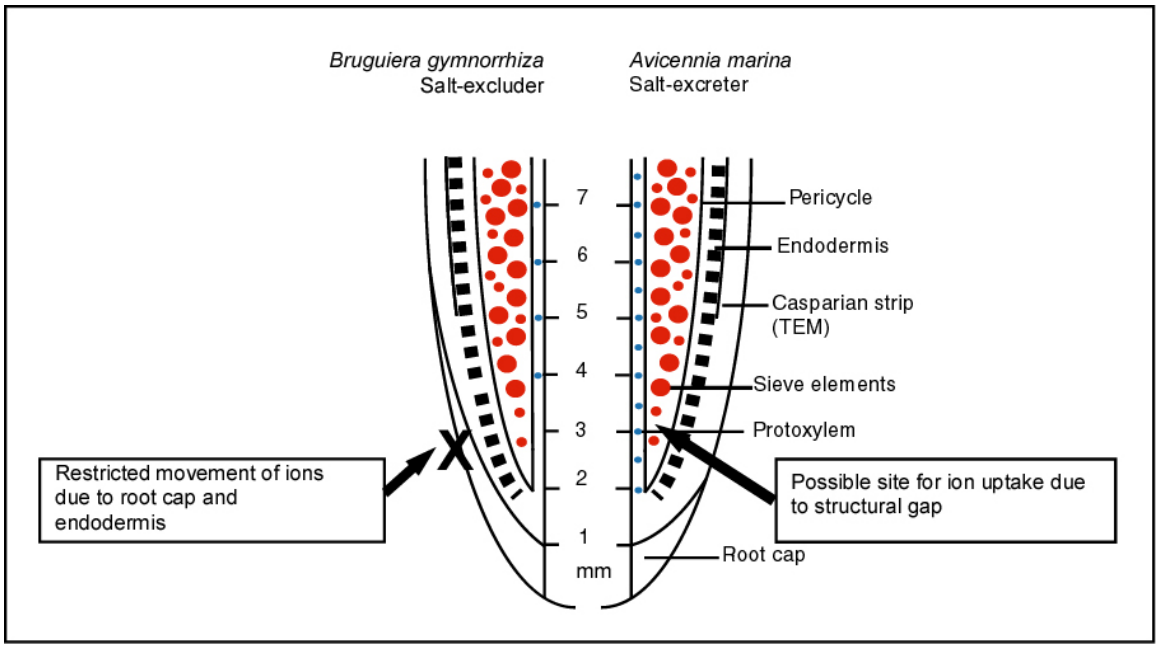

FIGURE 59: Root-tip structure of a salt-excluder, *Bruguiera gymnorrhiza* and a salt-excretor, *A. marina* (Lawton *et al.,* 1981).

For compounds other than salt, it has been found that *A. marina* has a greater uptake of oil (between two to six times) than *R. stylosa* and *R. stylosa mucronata* (Suprayogi and Murray, 1999). Resistance of *R. stylosa mangle* seedlings to oil may be due to the species' ability to exclude oil (Getter *et al.,* 1985). Heavy metals that reach leaf tissues in *A. marina* may be excreted in a similar way to other ions (MacFarlane and Burchett, 2000). In contrast *Bruguiera gymnorrhiza* accumulates only very small amounts of metals in leaf tissues, as most of the heavy metals absorbed are accumulated in the stem and root tissues instead (Yim and Tam, 1999). The endodermal gap in *A. marina* may also play a role in the uptake of herbicides.

The main objectives of this investigation were to;

- Determine if mangroves were affected by herbicides applied to their roots.
- Compare the response of mangrove seedlings with different physiologies (salt-excreting and salt-excluding species) to herbicide application.
- Rank the relative sensitivity of *A. marina* to other mangrove species.
- Rank the toxicity of diuron and related herbicides to mangroves.

This experiment was the first to quantify the effects of soil application of PSII herbicides on different mangrove species.

Methods

Plant Collection

Seedlings of the mangroves *A. marina*, *A. corniculatum*, *R. stylosa* and *C. australis* were collected from various locations in Moreton Bay, Queensland. Seedlings were potted in opaque plastic pots in a commercially prepared sediment mix consisting of 40% washed river sand, 30% peat (heat treated, toxin free, recycled pine) and 30% silty clay loam. The mangroves were left to adjust to conditions in the planthouse for 3-4 months prior to the experiment, exposed to natural day length conditions (Figure 68b).

Experimental Setup

Experiments were conducted in a planthouse located at the Moreton Bay Research Station, North Stradbroke Island, Australia (27°30'02"S 153°24'00"E) – see Figure 68a. Twelve individual tidal tank units were used, each consisting of two tanks; a lower water storage tank (~300L) and an upper tank, which contained the potted mangrove seedlings (Figure 60b). Saline water (16‰) was pumped up from the storage tank to the upper tank with a 12-volt bilge pump, for one-hour periods twice a day. Tanks were replenished with fresh water as needed, to compensate for evaporative loss. Slow-release fertiliser (NPK analysis 27:5.5:9 + trace elements) was added every 2 months in 500g doses to each tank system prior to the experiment. No fertiliser was added and the water was not changed in the tanks following the dosing with herbicides.

The twelve tanks were divided into four treatment groups (diuron, atrazine, ametryn and the control), with three tanks per group (Figure 60a). Prior to the experiment, 32 healthy mangroves (8 of each species) were selected and randomly assigned to each tank, with a total of 384 mangrove seedlings used. Due to height restrictions, mangroves were further subdivided into two groups; those with leaves above the high water mark (root exposure) and those with leaves below the high water mark (root and foliage exposure) – see Figure 60b. It was not fully appreciated until the experiment was underway that there might be a notable difference in the responses of these two groupings. They have been kept separate in the following assessment to avoid confusion. Each herbicide treatment was applied in four doses, with all four concentrations applied to separate pots in the same tank. Control tanks were treated with fresh water only.

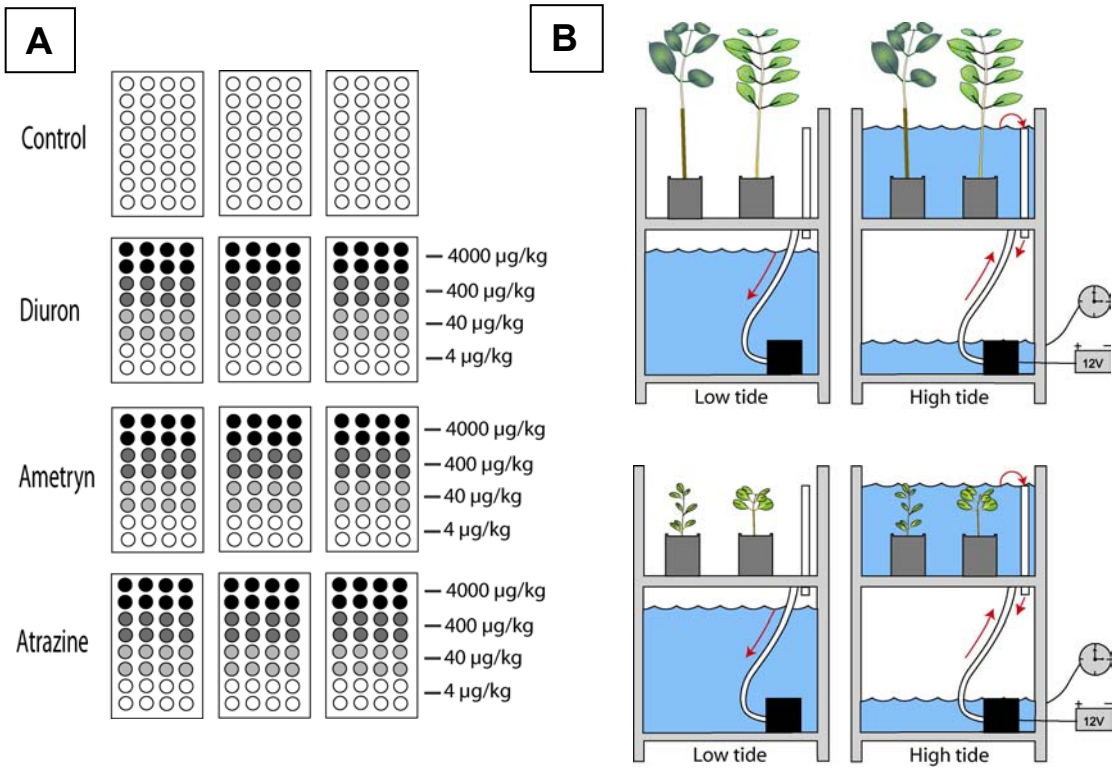

FIGURE 60: (A) Experimental design in the planthouse, consisting of 12 individual tidal tank systems, four treatment groups, and four concentrations of herbicides applied to four mangrove species. **(B)** Tidal tank set-up in the mangrove planthouse, showing different tidal regimes and problems associated with plant height. Taller trees (*R. stylosa* and *A. marina*) had leaves emerging above the high water mark while shorter trees (*A. corniculatum* and *C. australis*) had leaves submerged during the high tide.

Dosing

Herbicide solutions of atrazine (900g/kg pure, Atradex, WG ®), diuron (98% pure, Sigma ®) and ametryn (500g/L, Viking, SC) were prepared as a fresh solution for each dosing. Dosing solutions were made by first dissolving the herbicide in 5mL of acetone, then diluting it in 100mL of fresh water, followed by gentle heating to volatise the acetone. The solution was then made up to 2 litres with fresh water. Commercially bought clay (0.1 mg dw) was added to and mixed with each solution in order to simulate natural runoff conditions, where herbicides bind to sediment particles during runoff events. The solution was mixed constantly for 1 hour. A sterile syringe was used to extract 60 mL of herbicide solution from the container, which was applied evenly over the sediment surface of each pot as the tide was receding. Initial herbicide dosing concentrations were 4000, 400, 40 and 4 µg/kg dw of sediment for each herbicide. The sediment in each pot weighed approximately 1.7kg (dw), therefore the exact amounts of herbicide applied to each pot was 6800, 680, 68 and 6.8µg respectively. Exposure of mangroves to herbicides was the single dose to each pot at the start of the experiment, and no further doses were added. For the control samples, 5mL of acetone was diluted in 100mL of fresh water, and heated to volatise the acetone. The solution was made up to 2 litres with fresh water and 0.1mg dw clay was added and mixed with the water for 1 hour. Control plants were dosed with 60mL of the solution with a sterile syringe as the tide was receeding.

Sample Collection and Analysis

3 plants of each species (*A. marina*, *A. corniculatum*, *R. stylosa* and *C. australis*) were dosed with 4000µg/kg diuron, as an independent experiment in order to observe differences in diuron uptake in each species. Plants were harvested and leaves of *A. marina*, *A. corniculatum*, *R. stylosa* and *C. australis* were taken at day 11 (when the symptoms of herbicides were noticeable in *A. marina*)

after treatment with diuron. Leaves were frozen immediately and transported frozen for analysis at QHSS. The vegetation was blended at high speed with acetone. Dichloromethane was added to physically separate bulk water. The extract was then filtered through anhydrous sodium sulphate to remove residual water. This extract was then concentrated by rotary/vacuum evaporation and the solvent was exchanged to methanol/water for LC/MS/MS determination against prepared standards of the herbicides requested. Results were expressed in micrograms per kilogram (µg/kg) on a fresh weight basis.

Sediment samples were taken from the treatment pots at days 7, 14, 21 and 71 of each concentration from diuron, atrazine, ametryn and control treatments, to determine the actual concentrations of herbicides applied to the sediments, and the rates of herbicide degradation in the sediment. Samples were taken as a scoop from the top ~1cm of sediment from 6 pots with the same dosage concentrations. Samples were pooled for each treatment type, and were taken from pots with different mangroves species, based on the assumption that there would be no species effect on herbicide concentration, especially in the surface sediments. Samples were frozen immediately, and transported frozen, for analysis at QHSS. Solvent (acetone/hexane) was added to the sediment and shaken overnight on a mechanical shaker to allow for physical separation of bulk water. The extract was then filtered through anhydrous sodium sulphate to remove residual water. This extract was then concentrated by rotary/vacuum evaporation and the solvent was exchanged to methanol/water for LC/MS/MS determination against prepared standards of the herbicides requested. A separate sediment sub-sample was used to determine dry weight for calculation. The results were expressed in micrograms per kilogram (µg/kg) on a dry mass basis.

Water samples were taken at day 21 from each tank to determine the concentration of herbicide in the water. Samples were collected in 1L solvent-washed glass containers. These samples were analysed at the National Research Centre for Environmental Toxicology (ENTOX). The water samples were filtered prior to extraction. A glass microfibre filter (Whatman GFA 90 mm ∅) was rinsed with acetone then thoroughly rinsed with deionised water before being clamped in place over a scinter under a funnel. A water sample was added to the funnel and collected in a modified Schott bottle. The filtered water samples, were extracted by solid phase extraction (SPE) using an Oasis Extraction Cartridge, (Waters, HLB 12cc 500mg LP). SPE cartridges were conditioned with 5mL of methanol followed by 5mL of deionised water. The cartridge was fitted into a lid made for the Schott bottle with a seal. The Schott bottle was then inverted; a valve was opened in the bottom of the bottle and the water sample passed slowly through the cartridge. After the sample had passed through the cartridge, the Schott bottle was rinsed with 100mL of RO water, which was also extracted through the cartridge. Following the extraction of the water on to the solid phase, the cartridges were eluted with 10mL of methanol. One millilitre of methanol was first eluted through the cartridge, then discarded, to remove water held in the cartridge. The cartridges were fixed in place over clean 15mL calibrated test tubes and 10mL of methanol was added. Once the 10mL had eluted through, the sample was spiked with 50μL of dimethylsulfoxide (DMSO) as a keeper and were then reduced using a gentle stream of nitrogen to 0.5mL. The sample was then made up to exactly 1mL with deionised water and transferred to vials ready for analysis by LCMS. Results were expressed in micrograms per litre (µg/L).

Fluorescence Measurements

A Pulse Amplitude Modulated Fluorometer (Walz, Germany) was used to determine minimal fluorescence (F_o), maximum fluorescence (F_m) and maximum potential quantum yield (F_v/F_m). Chlorophyll fluorescence was measured using dark-adapting leaf clips, which maintain a constant distance between the fibre optic cable and the mangrove leaf. Leaves were dark adapted for 15 minutes. The fluorescence signal was measured at a standard position on the leaf (the middle of the adaxial surface on the second leaf pair from the plant apical meristem). Fluorescence measurements were made over a continuous time scale (daily) for 16 days, with day 1 being the first treatment, and then again at 71 days.

Statistical Analysis

Fluorescence data were taken from two separate plants of the same species and treatment concentration from the same tank for each measurement. These values were averaged, as they were not independent replicates. Prior to analysis, data were analysed for deviations from normality using a Kolmogorov-Smirnov test, and homogeneity of variance using Cochran's Q test. Data were log-transformed and re-evaluated where necessary. The data was analysed using a repeated measures analysis of variance (ANOVA) model, where there were three treatments (diuron, ametryn and atrazine), four treatment concentrations, 17 sample times (day 1-16 and day 71) and two mangrove species. Separate ANOVAs were performed for root only treatments (*A. marina* and *R. stylosa*) and root and foliage treatments (*C. australis* and *A. corniculatum*). Where the F-statistic was significant ($p<0.05$), a post-hoc Fischer's LSD (Least Squares Difference) test was used to locate significant differences in interactions between time, species, concentration and treatment. Where significant differences were expected between one or more pairs of means, but the F-statistic is non-significant, the Duncan's multiple range test was used. All statistics were carried out using the STATISTICA V6 software package. A one-way ANOVA was used to analyse differences in diuron uptake in the four different species of mangroves. A post-hoc Duncan's multiple range test was used to test significant differences between the means.

Results

Plant Responses

Tables 21 and 22 display the results of the Repeated Measures ANOVA for maximum potential quantum yield measurements (F_v/F_m) from mangrove plants. As the experimental period was short, significant F_v/F_m responses were only observed at the highest dosage concentration ($p<0.0001$) for both mangroves with root exposure to herbicides only, and those with both root and foliage exposure. Plants affected by herbicide treatment displayed characteristic chlorosis and necrosis along the mid-veins of the leaves (Figure 68 c, e + f). This was observed in all species affected by herbicides except for *A. corniculatum*, which in all cases only displayed chlorosis and wilting of leaves (Figure 68d). Plant height and growth rate were not affected by the herbicides in the short time period of the experiment.

For the species exposed to root-applied herbicides (*R. stylosa* and *A. marina*), significant main effects were seen for dosage concentration, species and time (days) (Table 21). There were also significant interactions between time x herbicide treatment x concentration x species, indicating significant variability in mangrove species response to different herbicides at the highest concentration over time.

TABLE 21: Summary of the repeated measures ANOVA of maximum potential quantum yield (F_v/F_m) of *R. stylosa* and *A. marina* over the entire experiment.

Effect	SS	df	MS	F-value	P
Herbicide treatment	52	2	26	1.78	0.179
Dosage concentration	2290	4	572.6	39.31	<0.001
Species	244	1	243.9	16.74	<0.001
Treat*Conc	441	8	55.1	3.78	<0.002
Treat*Species	87	2	43.7	3	0.059
Conc*Species	1284	4	321.1	22.04	<0.001
Treat*Conc*Species	307	8	38.3	2.63	<0.018
TIME	314	15	20.9	20.4	<0.001
TIME*Treat	48	30	1.6	1.56	<0.030
TIME*Conc	1047	60	17.5	17.02	<0.001
TIME*Species	137	15	9.1	8.91	<0.001
TIME*Treat*Conc	343	120	2.9	2.79	<0.001
TIME*Treat*Species	54	30	1.8	1.75	<0.009
TIME*Conc*Species	604	60	10.1	9.82	<0.001
TIME*Treat*Conc*Species	186	120	1.5	1.51	<0.001

For the species exposed to herbicides via roots and foliage (*A. corniculatum* and *C. australis*), significant main effects were seen for dosage concentration and time (days) (Table 22). There were also significant interactions between time x concentration x species, indicating significant variability in mangrove species response at the highest concentration over time.

TABLE 22: Summary of the repeated measures ANOVA of maximum potential quantum yield (F_v/F_m) of *C. australis* and *A. corniculatum* over the entire experiment.

Effect	SS	df	MS	F-value	p
Herbicide treatment	0.9	2	0.46	0.039	0.962
Dosage concentration	368.9	4	92.24	7.763	<0.001
Species	20.1	1	20.11	1.693	0.199
Treat*Conc	44.3	8	5.54	0.466	0.874
Treat*Species	62	2	31.02	2.61	0.084
Conc*Species	327	4	81.74	6.879	<0.001
Treat*Conc*Species	86.2	8	10.77	0.907	0.519
TIME	121	15	8.06	7.1	<0.001
TIME*Treat	42.2	30	1.41	1.238	0.18
TIME*Conc	262.3	60	4.37	3.849	<0.001
TIME*Species	50.2	15	3.34	2.945	<0.001
TIME*Treat*Conc	129.8	120	1.08	0.953	0.622
TIME*Treat*Species	43.2	30	1.44	1.268	0.155
TIME*Conc*Species	202.6	60	3.38	2.973	<0.001
TIME*Treat*Conc*Species	127.5	120	1.06	0.935	0.671

Diuron

Root exposure - Of the two species, *A. marina* was affected to the greatest extent by diuron (Figure 61a) and was significantly different from the control from day 16 ($p<0.05$). *R. stylosa* was not significantly different from the control throughout the entire experimental period although it did show a decline in health at day 71. Neither species showed any sign of recovery. Mortality occurred in both *A. marina* and *R. stylosa*, but was highest in *A. marina*.

Root and foliage exposure – Both *A. corniculatum* and *C. australis* were not significantly different from the control from day 1-16 (Figure 61b), although *A. corniculatum* did display a marked reduction in health. At day 71, all *A. corniculatum* were dead and *C. australis* was also unhealthy (although not significantly different from the control). Neither species displayed any signs of recovery.

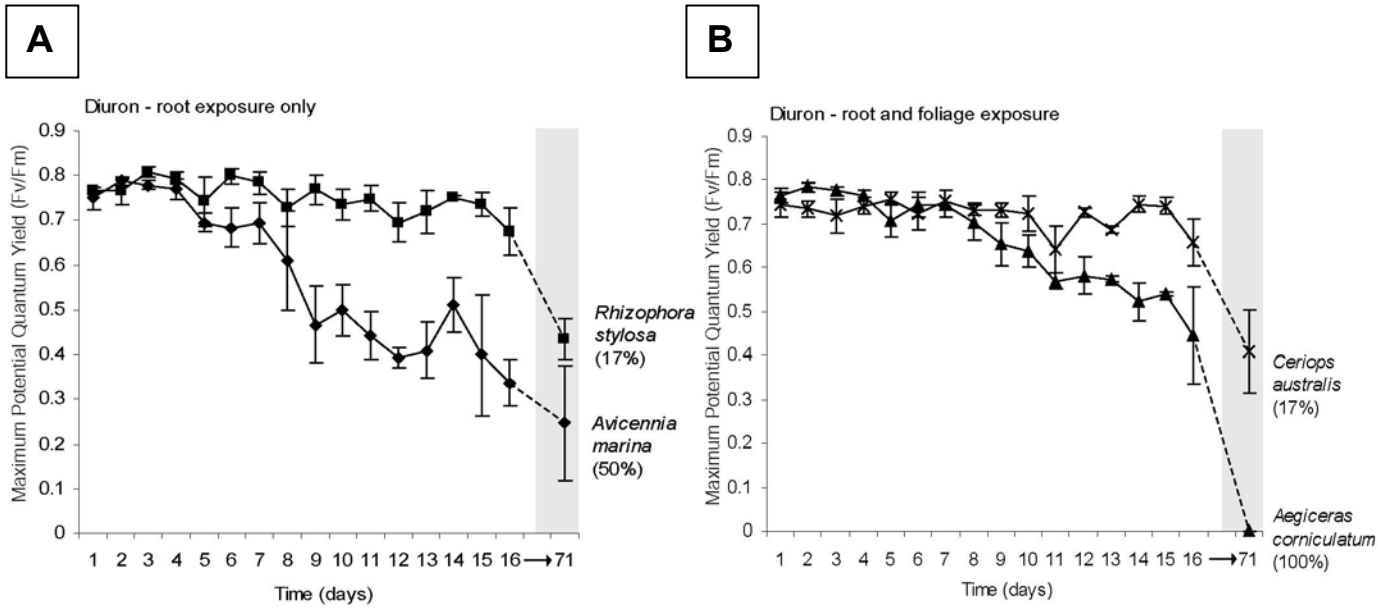

FIGURE 61: (A) Maximum potential quantum yield (F_v/F_m) responses of *R. stylosa* (■), and *A. marina* (♦) with root-only exposure to diuron at the highest dosage concentration (4000 µg/kg). **(B)** Maximum potential quantum yield (F_v/F_m) responses of *C. australis* (**X**) and *A. corniculatum* (▲) with both foliage and root exposure to diuron at the highest dosage concentration (4000 µg/kg). Units of maximum potential quantum yield are arbitrary (error = 1 SEM). Numbers in brackets indicate percent mortality (n=6 for each species).

Ametryn

Root exposure - (Figure 62a) *A. marina* and *R. stylosa* were not different from the controls throughout the entire experiment. In spite of this, *A. marina* showed physical symptoms of injury (chlorosis and necrosis) from the herbicide from early in the experiment and appeared to be recovering at day 71. There was a single *A. corniculatum* plant in the ametryn treatments with foliage above the high water mark. Although this plant appeared to be affected by ametryn at the start of the experiment, it recovered at day 71. There was no mortality of any species treated by root exposure only.

Root and foliage exposure – *A. corniculatum* was the only species to be affected by the ametryn treatments and was significantly different from the control after day 15 ($p<0.05$) (Figure 62b). Mortality of *A. corniculatum* was high.

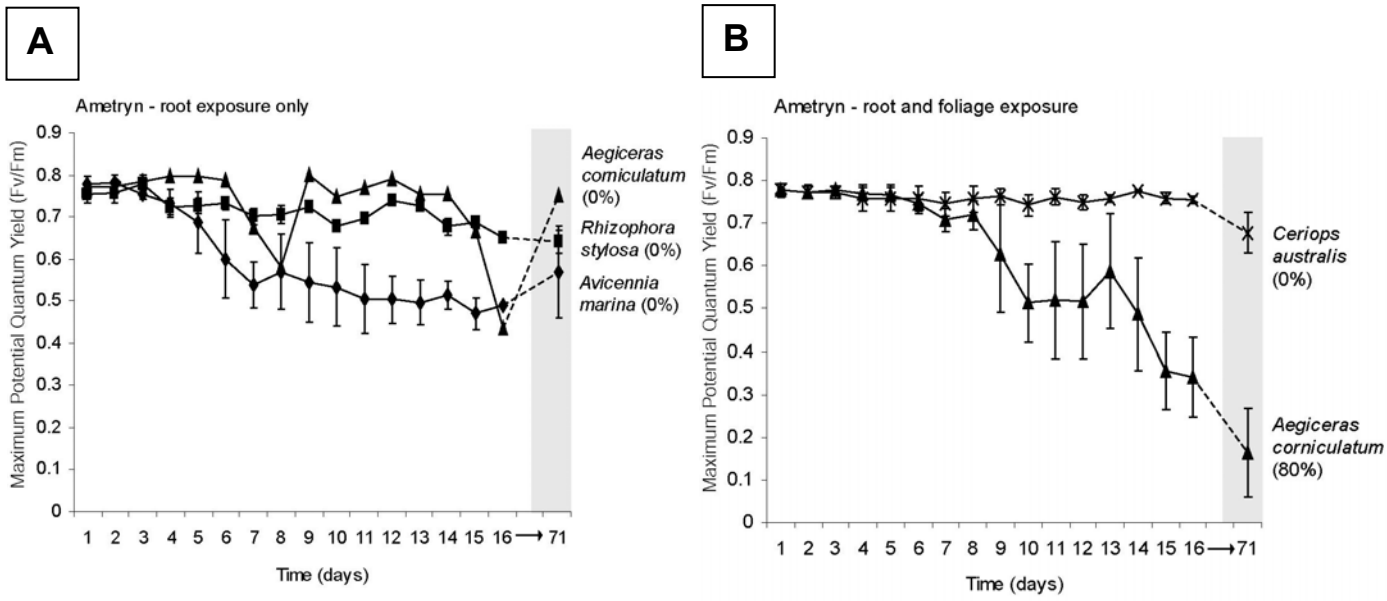

FIGURE 62: (A) Maximum potential quantum yield (F_v/F_m) responses of *R. stylosa* (■, n=6) *A. marina* (♦, n=6) and *A. corniculatum* (▲, n=1) with root-only exposure to ametryn at the highest dosage concentration (4000 µg/kg). **(B)** Maximum potential quantum yield (F_v/F_m) responses of *C. australis* (**X,** n=6) and *A. corniculatum* (▲, n=5) with foliage and root exposure to ametryn at the highest dosage concentration, (4000 µg/kg). Units of maximum potential quantum yield are arbitrary (error =1 SEM). Numbers in brackets indicate percent mortality.

Atrazine

Root exposure – Only *A. marina* was affected by atrazine (Figure 63a). Atrazine was the most rapidly working herbicide to affect *A. marina* of all three herbicides, being significantly different from the control after day 9 ($p<0.05$), compared with day 16 for diuron (Figure 64). *A. marina* plants that were affected by atrazine showed signs of recovery at day 71, with fluorescence values not significantly different from the control at this time. This recovery was characterised by the resprouting of stems and leaves from epicormic buds and an increase in photosynthetic activity in the plant. There was no mortality of *A. marina* or *R. stylosa* treated with atrazine.

Root and foliage exposure – Neither *A. corniculatum* or *C. australis* were affected by atrazine throughout the experiment (Figure 63b). There was no mortality of either species treated with atrazine.

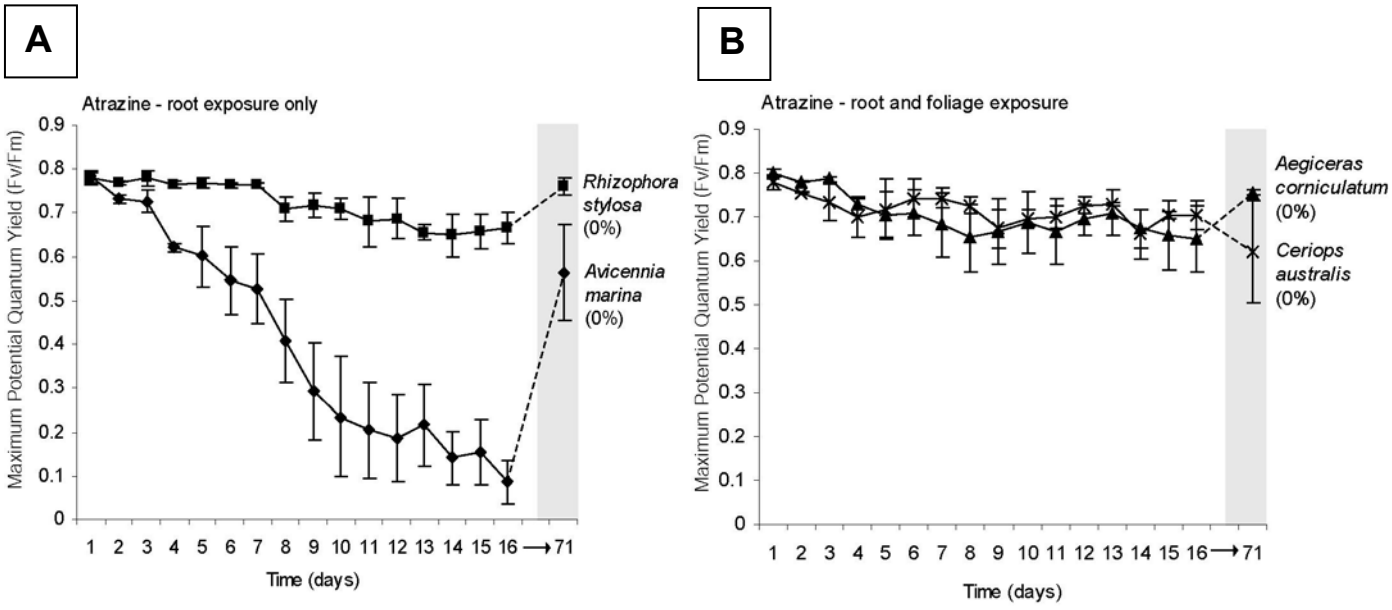

FIGURE 63: (A) Maximum potential quantum yield (F_v/F_m) responses of *R. stylosa* (■) and *A. marina* (♦) with root-only exposure to atrazine at the highest dosage concentration, (4000 µg/kg). **(B)** Maximum potential quantum yield (F_v/F_m) responses of *C. australis* (**X**) and *A. corniculatum* (▲) with both foliage and root exposure to atrazine at the highest dosage concentration, (4000 µg/kg). Units of maximum potential quantum yield are arbitrary (error =1 SEM). Numbers in brackets indicate percent mortality (n=6 for each species).

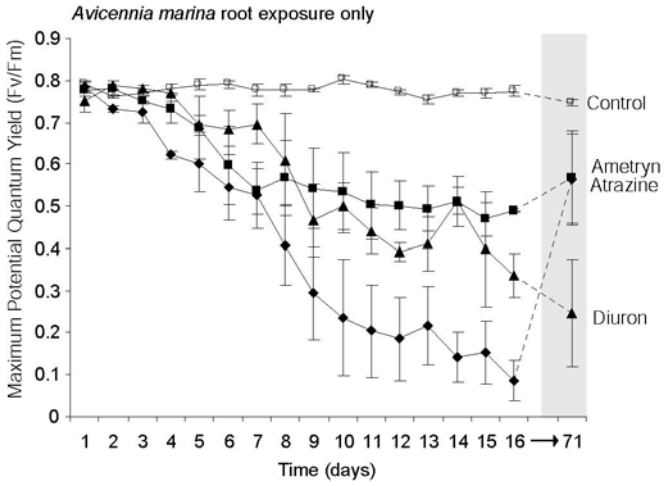

FIGURE 64: Maximum potential quantum yield responses of *A. marina* in control (O) tanks, and with exposure to diuron (▲), atrazine (♦) and ametryn (■) at the highest dosage concentration (4000 µg/kg). Units of maximum potential quantum yield are arbitrary (error =1 SEM).

Diuron Uptake in Mangroves

The salt-excreting species, *A. marina* had a significantly greater ($p<0.05$) uptake of diuron per kg of plant mass than *R. stylosa* (Figure 65a). Diuron uptake in submerged *A. corniculatum* was significantly greater than uptake in *C. australis* (Figure 65b).

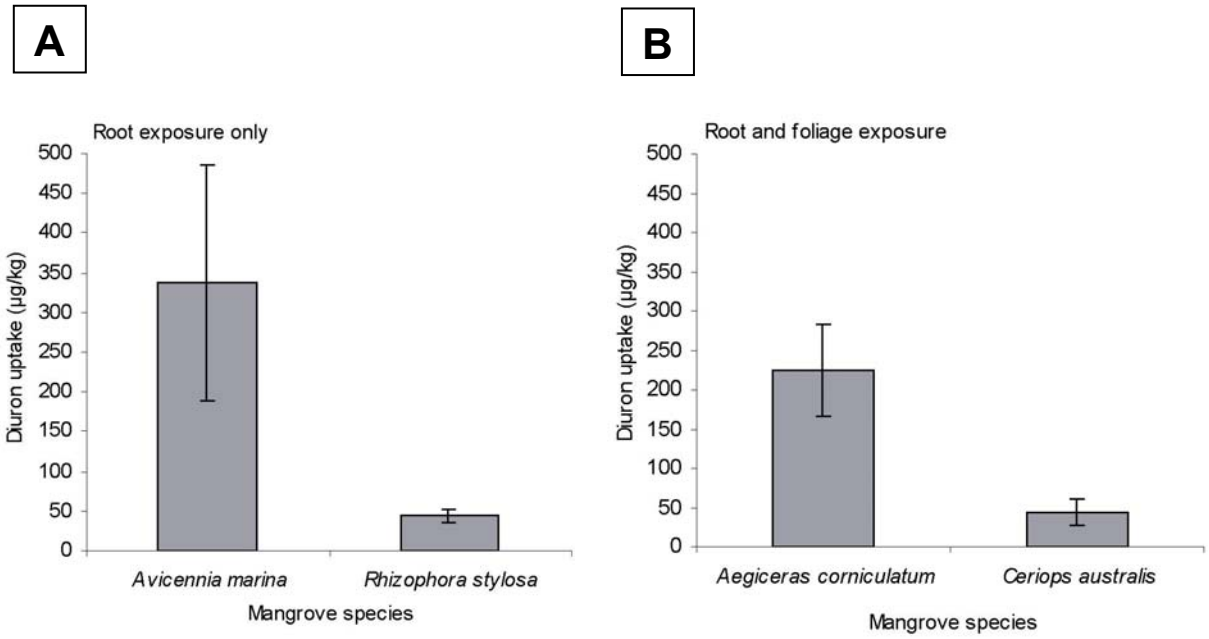

FIGURE 65: Diuron uptake in mangrove leaves (µg/kg) after 11 days of exposure to 4000 µgkg dosage of diuron in **(A)** root-exposed plants only (*A. marina* and *R. stylosa*) **(B)** root and foliage exposed plants (*A. corniculatum* and *C. australis)* (error term = 1 SEM). Reporting limits for diuron and atrazine were 0.1µg/kg, and ametryn, 0.05µg/kg on a dry weight basis.

Herbicide Concentration and Degradation

Water Concentration

All concentrations of herbicides in the water were taken at day 21. It should be noted that these values represent the herbicides that did not adhere to the sediment of the pots and as there were a number of different application concentrations within each tank, the water concentrations are a composite of these leached herbicides leached from the pots. The concentration of diuron in the water from the tidal tanks was 13.73 µg/L (±1.39 SE), ametryn was 7.75 µg/L (±1.7 SE) and atrazine was 7.71 µg/L (±2.29 SE).

Sediment Concentration

These results demonstrated vast differences between actual herbicide concentrations applied and concentrations measured, which were calculated to be up to three times the amount applied for diuron and ametryn at day 7. The concentrations also were quite variable between sampling periods (Table 23).
Figure 66 displays the degradation patterns of diuron, ametryn and atrazine from day 7 to day 71 of the experimental period. Degradation was calculated from the total concentration of all pots for each treatment, to normalise for the unintended transfer of herbicides between pots after application. It was not practical to calculate degradation from the initial dosage concentrations (day 1) because after the application to the sediment, the herbicides are likely to have displayed different rates of movement through the sediment profile, which would have influenced the actual concentration measured at the top of the soil profile where the samples were taken. This is why it is not feasible to say that atrazine had the fastest degradation from application time to day 7, even

though it appears to (Figure 66). Because of this, degradation was calculated from day 7 to day 71 of the experiment. Diuron and ametryn degraded slower than atrazine over this time period. At day 71, the total concentrations of diuron and ametryn had degraded to 23% and 21% respectively of the day 7 concentrations, while for atrazine the concentrations were at 3% of the day 7 levels.

TABLE 23: Herbicide concentrations (µg/kg) of diuron, ametryn and atrazine in the top ~1cm of the sediment in pots, in the planthouse trials. Samples were pooled from 6 pots of the same concentration (2 from each tank). Reporting limits for diuron and atrazine were 0.1µg/kg, and ametryn, 0.05µg/kg on a dry weight basis.

Initial dosage concentrations ($\mu g\ kg^{-1}$)	**Actual measured concentrations ($\mu g\ kg^{-1}$ dw)**			
DIURON ($\mu g\ kg^{-1}$ dw)	**Day 7**	**Day 14**	**Day 21**	**Day 71**
4000	12000	8500	5100	2000
400	1200	4600	780	600
40	830	690 *	550	500
4	170	290 *	410	198 *
Control	ND	ND	ND	ND
AMETRYN ($\mu g\ kg^{-1}$ dw)	**Day 7**	**Day 14**	**Day 21**	**Day 71**
4000	12000	5000	6200	2200
400	1900	1100	310	640
40	60	57 *	53	92
4	20	19*	17	8 *
Control	ND	ND	ND	ND
ATRAZINE ($\mu g\ kg^{-1}$ dw)	**Day 7**	**Day 14**	**Day 21**	**Day 71**
4000	2700	670	400	61
400	190	32	580	8
40	49	36 *	22	8
4	34	26 *	17	1*
Control	ND	ND	ND	ND

* Values were extrapolated from existing data
ND indicates no detection of herbicide

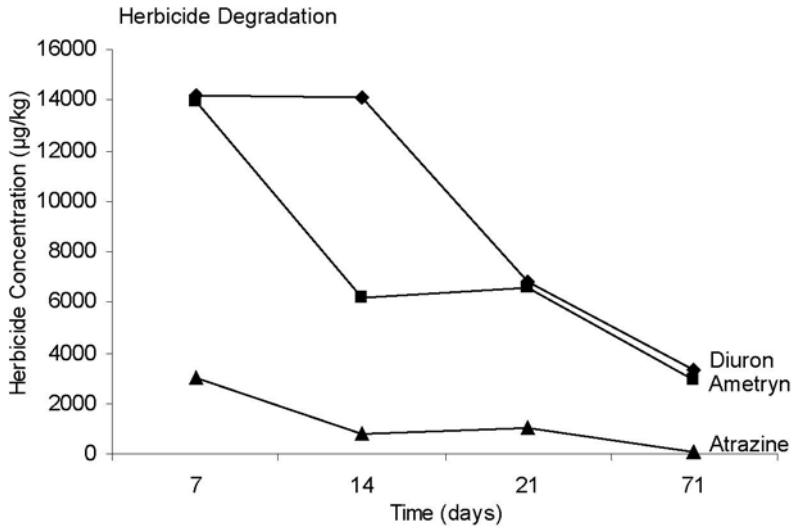

FIGURE 66: Total calculated concentrations of diuron, ametryn and atrazine (µg/kg) in sediments in the top 1cm of the sediment from pots in the planthouse experiments. Concentrations displayed are a total of all concentrations in the tanks, to calculate degradation rate. This was to normalise for accidental transfer between pots with different doses. Reporting limits for diuron and atrazine were 0.1µg/kg, and ametryn, 0.05µg/kg on a dry weight basis.

Discussion

Studies on the relationships between herbicides and mangroves are few, and focus on less frequently used, or banned herbicides such as 2,4-D (Westing, 1971; Walsh *et al.,* 1973; Walsh *et al.,* 1974; Culic, 1984). There are apparently no publications regarding the root-uptake of the more prevalent herbicides currently in use, especially Photosystem II-inhibiting herbicides. These trials are the first to address this issue. In the current investigation, PSII herbicides were applied in a range of concentrations, beyond those measured in the field. This was done to ensure a rapid response, if any, as a preliminary review of relative impacts. It was also our objective to achieve a result in a relatively short time period.

The results demonstrate that mangroves were affected by PSII-inhibiting herbicides as plants in control tanks were clearly not affected throughout the experiment. The response however is different depending on the species, its methods of salt-regulation, and the method of application of the herbicide. This experiment showed that regardless of whether plants were exposed to herbicides through their roots only or by both roots and foliage, that salt-excreting mangroves (*A. marina* and *A. corniculatum*) were more vulnerable than the salt-excluding species, for the four herbicides tested. Both *A. marina* and *A. corniculatum* had the highest uptake of the herbicide diuron in the short-term (11 days). Salt-excreting taxa, especially *A. marina*, also reportedly have greater uptake of other compounds, including oil (Getter *et al.,* 1985; Suprayogi and Murray, 1999), heavy metals (Lacerda, 1998) and sewage-derived nitrogen (δ15N) (Duke *et al.,* 2001) – See Figure 67. As root uptake is the main route for transport of herbicides used in this experiment, (Chambers, 1997), it seems likely that the same mechanism that regulates salt influx in mangroves, i.e. root morphology, also regulates the uptake of other compounds, including herbicides. Future studies could focus on the susceptibility of other salt-excreting mangrove species, such as *Aegialitis* and *Acanthus*.

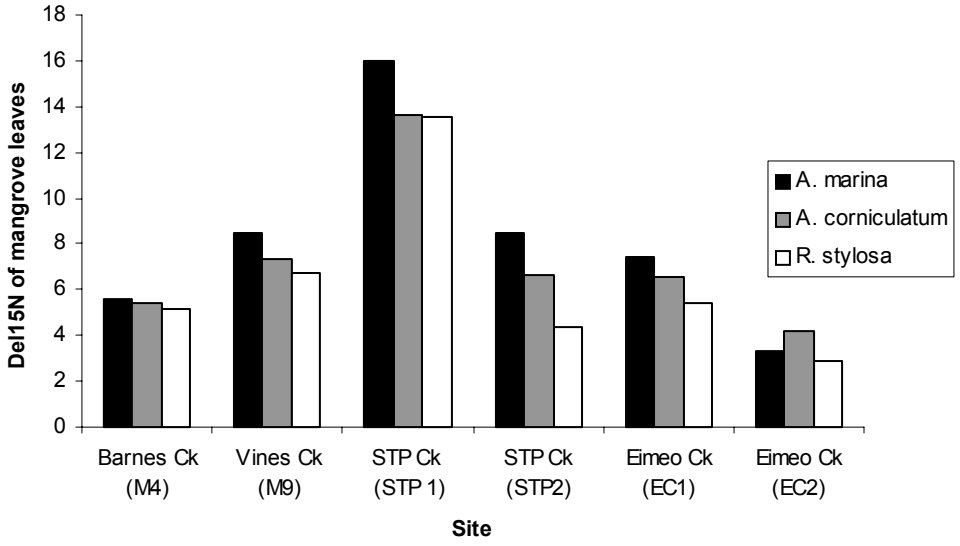

FIGURE 67: Del15N uptake in leaves from *A. marina*, *A. corniculatum* and *R. stylosa* from various locations in the Pioneer River, Mackay, Australia (Duke *et al*., 2001).

Within the grouping of salt-excreters, *A. marina* and *A. corniculatum* displayed different vulnerability to the herbicides, with variations in both the time taken to respond to the herbicides, and long-term results. Generally *A. marina* was affected first and to the greatest extent by all three herbicides, however *A. corniculatum* was affected to a greater extent by the diuron and ametryn treatments in the long-term. This result is likely to have been influenced by the fact that *A. corniculatum* was submerged during periods of high tide. There is some capacity for translocation of these herbicides through the foliage (Percival and Baker, 1991). Movement of most herbicides through the leaf is a passive diffusion process, and depends on the physicochemical properties of the herbicide and the epicuticular wax layer on the leaf surface (Percival and Baker, 1991). In

these experiments, all unhealthy and dead *A. corniculatum* seedlings were below the high tide level (no foliage was above the water). Healthy *A. corniculatum* trees were either only found at low concentrations of herbicide treatment, or when plants had foliage that was not submerged, such as in the ametryn treatment. There was a constant concentration of herbicide in the water flushing through the pots with every high tide and although initial water concentrations are not known, these concentrations alone were not high enough to affect mangroves (no seedlings were affected at the lowest concentrations of herbicides applied to the sediment). In spite of this, the cumulative effect of herbicide exposure to both leaves and roots may have exerted an added stress on the *A. corniculatum* seedlings making them more vulnerable to the herbicides. The only physical symptoms of injury in *A. corniculatum* were chlorosis and wilting of the leaves (Figure 68D), without showing any signs of necrosis, as did the leaves of all other species affected by the herbicides (Figure 68C, E and F). It therefore seems likely that the submergence of the *A. corniculatum* seedlings during high tide may have contributed to its demise in these trials. From the results with *A. corniculatum* in the ametryn treatment we could speculate that if *A. marina* seedlings were also submerged at high tide, they would be more vulnerable than *A. corniculatum* in the long-term. *C. australis*, the other species that was submerged at high tide, was not affected by any herbicide except for the highest concentration of diuron. Its comparatively resilient nature might be attributed to its salt exclusion properties, but also the cuticular properties of the leaf, which may have prevented the facilitation of herbicide movement through the leaf. In these experiments, foliage immersion was not specifically tested for, and based on current findings, this needs to be included in future experiments. Experimental design could involve raising shorter plants onto platforms, to eliminate variables associated with submergence.

Measurements taken of herbicide concentration, notably diuron and ametryn, in the sediments indicated that the actual concentration was up to 3 times that actually applied, and furthermore, extremely patchy. Diuron and ametryn are hydrophobic and adhere easily to organic matter in the sediment (Wauchope *et al.,* 1992) (Table 24). This is an important function of these herbicides, which allows them to remain on field after application. Because of these properties, it is likely that after application in the planthouse experiments, the herbicides diuron and ametryn accumulated in the top few centimetres of the sediment profile instead of distributing evenly throughout the sediment. While it was not possible to measure the concentration of herbicides in the lower region of the sediment throughout the experiment without disturbing the roots, results suggest that this accumulation did occur. Future experimental manipulations with planthouse trials may include testing different soil types including sand and clay to determine whether this affects the distribution of the herbicides in the sediment, and subsequent mangrove survival.

TABLE 24: Half-life and movement rating of the herbicides diuron, ametryn and atrazine, used in the preliminary toxicology trials (Wauchope *et al.,* 1992).

	Diuron	**Ametryn**	**Atrazine**
Half-life (days)	90	60	60
Herbicide movement rating	Moderate	Moderate	High

Of the three herbicides used in this experiment, diuron was considered to be the most toxic herbicide due predominantly to its slow degradation rate and also to its toxicity to all mangrove species. None of the species at day 71 treated with diuron showed any signs of recovery. Ametryn, having shown similar degradation rates to diuron, could also be considered to be one of the most toxic, however it did not affect all species as diuron did at the same concentrations. Furthermore, the photosynthetic inhibition caused by ametryn in affected plants was not as severe as diuron. Atrazine, on the other hand, was the least hydrophobic of the three herbicides, and would be expected to distribute through the sediment profile more evenly. The concentration of atrazine in the sediment was similar to what was expected, compared with diuron and ametryn. Atrazine was the fastest working and most photosynthetically damaging of all herbicides used in the short-term, possibly due to a faster delivery to the roots as a consequence of its higher water solubility than diuron and ametryn (Table 24). In spite of this, atrazine degraded rapidly, demonstrating less of a possibility for any long-term damage to the plants. There may be an opportunity for mangroves to

recover from herbicide damage in locations where the herbicide concentration in the sediment degrades to below a threshold level, however very little is known about the persistence of herbicides in mangrove sediments. Despite the fact that the dissipation rates of modern herbicides are usually quite rapid (Wauchope *et al.,* 1992), diuron has been shown to be highly persistent in red ferrosol soils (Simpson *et al.,* 2001b), being incorporated into the soil matrix enough to prevent normal chemical and microbial breakdown processes.

There may be further interactions and synergistic effects of environmental conditions with herbicides that have not been explored. It will be of significant value to learn how the manipulation of nutrients and salinity levels to mimic runoff events might affect the impact of different herbicides on mangroves.

Given the state of *A. marina* mangroves in the Mackay region, coupled with the presence of diuron in the mangrove sediments (Duke *et al.,* 2001; Duke *et al.,* current investigation), this experiment supports the suggestion that herbicides are a major factor contributing to dieback in the Mackay region. *A. marina* is usually a tolerant species, having a distributional range exceeding that of all other mangrove species in the Indo West Pacific (Duke *et al.,* 1998). It has also been shown to be quite resilient to physical damage such as storms and cutting, and regenerates by resprouting rapidly (Wadsworth, 1959; Tsuda and Ajima, 1999). In its response to environmental contaminants however, *A. marina* could be considered to be an 'indicator' species, displaying the early warning effects of these kinds of environmental stresses before they are apparent in other species and neighbouring ecosystems such as seagrasses and corals.

There is the clear need for similar experiments focusing specifically on dose-response assessment, and long-term effects on *A. marina*, using the results from these preliminary planthouse trials. How these herbicides affect mature mangrove trees as opposed to seedlings is also highly important. It is recommended that a controlled field study be conducted, such as dosing mature mangrove trees with specific herbicides within an enclosure.

Conclusions

The results from this study have demonstrated that

- Mangroves were affected by herbicides.
- There was a differential response to herbicides by salt excreting and salt-excluding species, with the salt-excreting species being more susceptible
- Overall, *A. marina* was likely to be the most sensitive species to herbicide application
- Diuron was likely to be the most toxic herbicide due to its slow degradation rate and ability to affect more than one species.

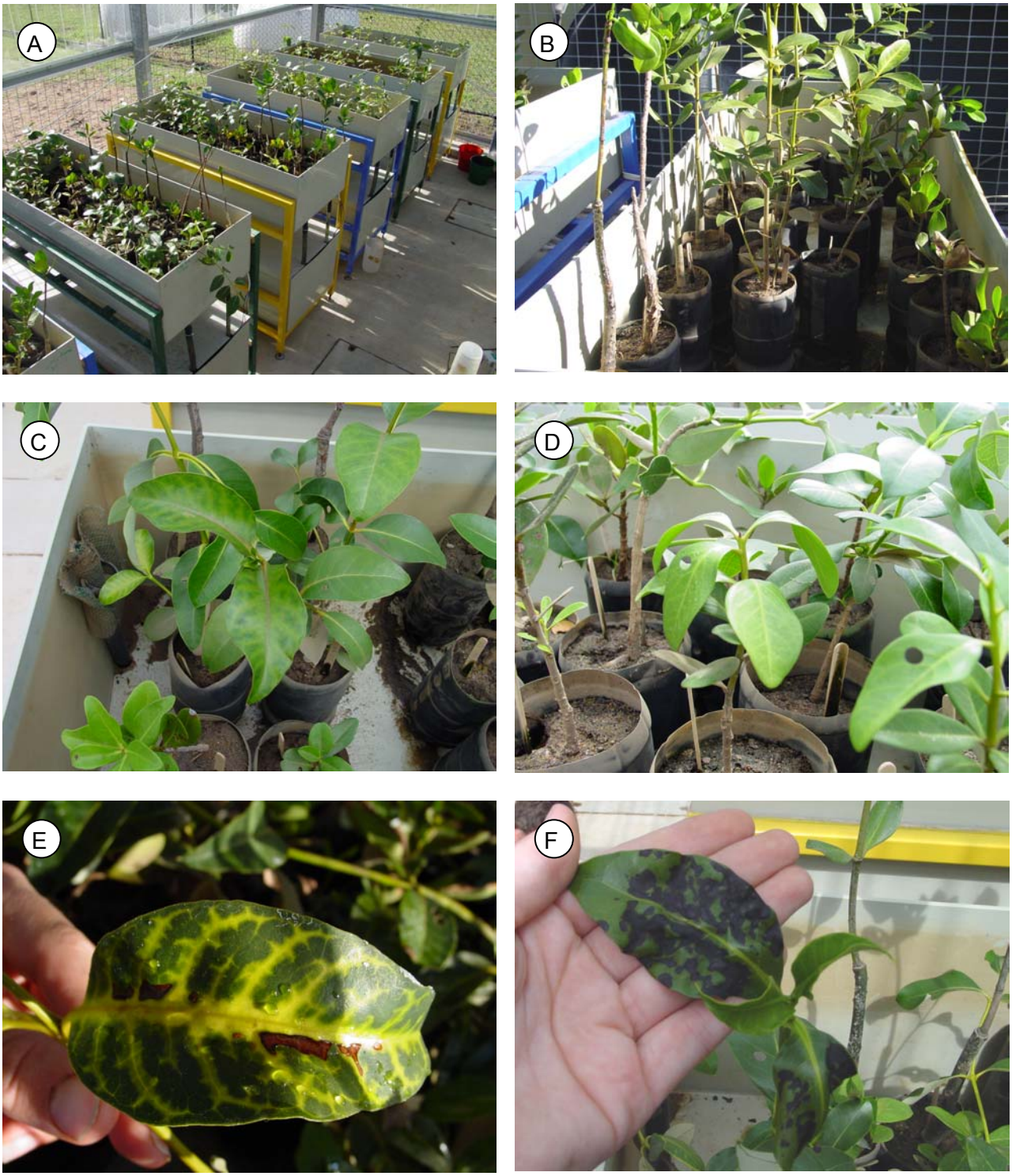

FIGURE 68: Planthouse studies at Moreton Bay Research Station **(A)** Tank set-up in the Planthouse **(B)** Seedlings with *A. marina* showing chlorosis **(C)** Chlorotic *A. marina* in the early stages **(D)** Chlorosis in *A. corniculatum* and wilting of leaves **(E)** Later stages of chlorosis in *A. marina* and beginning stages of necrosis **(F)** Necrosis in *A. marina.*

Comparative River Studies Assessing *Avicennia* and Herbicide Presence -

Johnstone and Daintree Rivers

Introduction

Two northern rivers, the Johnstone and Daintree Rivers (Figure 1) were chosen to compare mangrove composition (especially *A. marina*), distribution, health and condition in these estuaries to that of the Mackay region.

The Johnstone River was chosen specifically because it was comparable with the Pioneer River, in terms of it having significant modification and land allocated to agriculture. Two major tributaries comprise the Johnstone River catchment (17.509^{o}S 146.066^{o}E), the North and South Johnstone Rivers, which converge into a common estuary at the town of Innisfail and drain into the Coral Sea (Figure 69). The source of both tributaries is the south-eastern section of the Atherton Tableland. The catchments of the North and South Johnstone Rivers contribute approximately 2.7 million megalitres to the total discharge from the Johnstone Basin (Johnstone Shire Council, 2001). Current environmental issues in the catchment include erosion from grazing land and cropping land, lost habitat and threatened species, loss of coastal wetlands and high contribution of nutrients and pesticides from cropping lands (GBRMPA, 2001b). The Johnstone River has extensive areas of flood plains and coastal areas allocated to cultivation, especially sugar cane (394km^2) and horticulture (44km^2) (GBRMPA, 2001b). In 1996 (Hamilton and Haydon, 1996) diuron usage in the Johnstone River was reported at 17353 kg ai/yr while in the Pioneer River, it was 23435 kg ai/yr. In a survey of sub-tidal sediments along the Queensland coast in 1998, Haynes *et al*. (2000a) detected the highest levels of diuron (10.1 and 9.8 µg/kg dw) in the sediments of the Johnstone River out of 24 other sampling sites.

The main objectives of the Johnstone River study were to:

- Describe the structure and condition of mangrove forest plots (species, density, health and demography), and establish selected plots where a series of sediment, water and vegetation samples could be gathered
- Collect sediment, biota and water samples for analysis of possible contaminants, particularly pesticides, from a specific selection of sites throughout the Johnstone River
- Compare the presence of toxic substances in mangrove plots with *A. marina* and without *A. marina*

The Daintree River catchment (16.291^{o}S 145.451^{o}E) is located in tropical north Queensland and is of similar size to the catchment to the Johnstone River (2192 km^2 and 2325 km^2 respectively). The Daintree River drains a small coastal plain before discharging into the Coral Sea (Russell *et al.,* 1998). In contrast to the Johnstone and Pioneer River catchments, the much of the Daintree River catchment is pristine, with limited sugar (48 km^2) and grazing (45 km^2) (GBRMPA 2001c).

The main objective of this study was to:

- Determine the concentrations of herbicides from mangrove sediments in a river system with minimal agricultural inputs and healthy *A. marina* stands (Figure 72D).

Methods – Johnstone River

Fieldwork in the Johnstone River was conducted on the 24-29 November 2001. Sampling took place in both the North and South Johnstone Rivers as well as in the estuarine section towards the mouth. There were 7 mangrove plots and 9 water stations located in the river. For location of sites, refer to Figure 69.

Mangrove Identification, Density and Condition

In each plot, all mangrove species were identified and classified as healthy, unhealthy (sick) or dead. Unhealthy trees were visually characterised as having relatively low foliage density and yellow, wilting leaves. In order to determine stand structure, species identification, height (estimation where not possible to measure) and circumference of all trees in the plot were measured (Figure 72C). Seedling demography was measured in the same plot as the stand structure measurements (seedlings were identified as mangrove trees <0.5m tall). The following parameters were recorded; node count, height, circumference and leaf number.

Water Quality

Water Quality Parameters

Water sampling was conducted at high tide during the middle of the day (Figure 72B). Water clarity was determined by lowering a 30cm diameter secchi disk (with black and white alternating quarters) through the water column, until it was no longer possible to distinguish between the black and white sections. The depth at which this occurs is called the secchi depth.

The physical properties of the water column were tested for pH and temperature (^{o}C) using a TPS 90-FL instrument at each site. The TPS field instrument was calibrated according to the TPS 90-FL instruction manual. Data was collected by inserting the probes into the water and taking the reading when it had stabilised. Salinity was measured with a portable refractometer by adding a few drops of water onto the lens, and obtaining the reading through the adjustable eyepiece. Instruments were cleaned between sample sites to prevent cross contamination of the samples. The range(s), resolution and accuracy of each measurement are displayed in Table 3.

Chlorophyll

Samples were collected for chlorophyll in the water column in 2 litre plastic bottles. Samples bottles were rinsed three times before water collection and water was collected as a sub-surface grab sample (approximately 20cm below the water surface to avoid surface scum). Samples were kept cold in the field and then filtered through a 0.45 µm Whatman GF/C glass microfibre filter paper using a pump and filter towers. The filter papers were wrapped in aluminium foil and frozen. In the lab, the frozen filter paper was ground using a mortar and pestle and made up to 10mL in a centrifuge tube. Samples were stored in the freezer (-16^{o}C) to allow for pigment extraction for 24 hours and shaken once during this period. Samples were centrifuged for 20 minutes at 2000 rpm. 1 mL of solution was transferred using a glass pipette to a glass cuvette (1cm path length), and wavelengths were determined on a Pharmica LKB Ultrospec III Spectrophotometer. Wavelengths chosen for analysis were 647, 664, 610, 510, 480 nm. Chlorophyll analyses included determination of chlorophyll *a*, chlorophyll *b*, carotenoids, total chlorophyll (*a*+*b*) and ratio of chlorophyll *a*:*b*.

Sample Collection

Samples were taken for analysis of herbicides and heavy metals in the mangrove sediments. Sediment samples were taken as a pooled sample from five sub-samples (0-2 cm depth)

throughout the plot (Figure 72A). All samples for heavy metals, nutrients and pesticides were analysed at QHSS.

Nutrients

In each plot, 5 holes were dug in the mangrove sediment approximately 30x30cm and 30cm deep until there was sufficient water in the hole for sampling. From the core water in each hole, samples for nutrients (Filtered and Unfiltered) were collected

Soluble (filtered) water samples were collected in 100mL Reverse Osmosis Water washed plastic bottles. A 60mL sterile TERUMO disposable syringe was used to collect the water sample, which was filtered using a SARTORIUS "Minisart" hermetically sealed filter with 0.45µm pore size. The syringe was rinsed twice with sample water and filled completely with sample water (approximately 60mL) and filter was attached. Approximately 10mL of sample was filtered in to the sample bottle, bottle was capped, shaken and sample discarded, repeated. The remaining 40 mL of sample was filtered into the bottle. Filters were replaced when necessary. Bottle was capped, leaving 2cm airspace. Samples were kept cold in the field and frozen when possible. Samples were transported frozen, and analysed within 1 month of collection at QHSS for Phosphorus (total dissolved and filterable reactive phosphorus) and Nitrogen (ammonia, oxides and total dissolved nitrogen) in accordance with the methods of Clesceri *et al.* (1998) using a Skalar autoanalyser (Norcross, Georgia, U.S.A.).

Total (unfiltered) water samples were collected with 60mL TERUMO sterile syringes in 250mL Reverse Osmosis Water washed plastic bottles. Samples were kept cold in the field and frozen when possible (within 12 hours). Samples transported frozen and were analysed within 1 month of collection at QHSS for Total N and Total P in accordance with the methods of Clesceri *et al.* (1998) and Hosomi and Sudo (1986) using a Skalar autoanalyser (Norcross, Georgia, U.S.A.).

Heavy Metals

Samples of sediment for heavy metal analysis were collected in a 375mL acid washed glass container from a mixture of 5 sub-samples over the plot. These samples were taken from surface sediment, ie. to depths of ~5 cm. Sediments samples were analysed for Arsenic (As), Chromium (Cr), Copper (Cu), Iron (Fe), Manganese (Mn), Nickel (Ni), Lead (Pb), Zinc (Zn), Cadmium (Cd) and Mercury (Hg). Samples were kept cold in the field and frozen as soon as possible. Samples were transported frozen to QHSS for analysis. As, Cr, Cu, Fe, Mn, Ni, Pb and Zn were analysed by Acid Digestion (USEPA 3050), Cd was analysed by Inductively Coupled Plasma Atomic Emission Spectrometry (ICPAES) and Hg was analysed by Flame Atomic Absorption Spectrometry (FAAS). The samples were prepared to AS4479 and then crushed in a zirconia tema swing mill. The analysis was carried out on the fully crushed sample. The results for heavy metals are reported in micrograms per kilogram (µg/kg) on a dry mass basis.

Pesticides

Sediment

Samples of sediment for pesticide analysis were collected in a 375mL solvent-washed glass container from a mixture of 5 sub-samples over the plot. These samples were taken from surface sediment, i.e. to depths of ~5 cm. Sediment samples were analysed for a range of pesticides known to be in use in the Johnstone River Catchment. These included diuron, atrazine, ametryn, chlorpyrifos, DDE and 2-4D.

Sediment (approximately 50g) was weighed accurately into extraction vessel. Acetone (50mL) and Hexane (50mL) was added and shaken overnight on a mechanical shaker. Solvent was separated from the sediment using a centrifuge and the solvent was back extracted with 100mL dichloromethane. The extract was Rotary Evaporated to 7mL and run through a GPC Column to clean up the extract. Extract was concentrated to 1mL and run on GCMS. Following GCMS

analysis the extract was diluted to 1.5mL. Of this, 0.5mL was run on the LCMSMS and 1mL was further cleaned up on florisil before analysis on GCECD and GCFPD. The concentrations of pesticides were expressed in micrograms per kilogram (µg/kg) on a dry mass basis.

Vegetation

Within each plot leaf samples were collected for pesticide analysis from a range of mangrove species in the plot, to include both salt excreting and salt-excluding mangrove species. Leaves collected were fully expanded new leaves without notable damage. Leaf samples were washed with deionised water and frozen until analysis. Concentrations of pesticides are expressed in micrograms per kilogram (µg/kg) on fresh mass basis.

Approximately 5.0 g of leaves were weighed accurately into extraction vessel. Acetone (80mL) was added and extracted using a blender. Solvent was separated from the sample and extraction was repeated using 50mL of acetone and 50mL of hexane. The second extract was added to the first and concentrated on a rotary Evaporator. The extract was Rotary Evaporated to 7mL and run through a GPC Column to clean up the extract. Extract was concentrated to 2mL. 1mL was run on the LCMSMS and 1mL was further cleaned up on florisil before analysis on GCMS, GCECD and GCFPD.

Methods – Daintree River

A detailed analysis of mangrove species composition for trees and seedlings was not undertaken in the Daintree River as with the Johnstone and Mackay Rivers. Instead, data previously collected in the area (Duke, unpublished data) on species distribution in the river were combined with field observations of mangrove species distribution in the area.

Sediment

Sampling was conducted on the 22 June, 2002. Sediment samples were taken from mangrove sediments at three sites situated along the Daintree River (Refer to Figure 70 for location of sites). Sediment samples were taken as a pooled sample from five sub-samples (0-2 cm depth) in the area. Samples were kept cold in the field and frozen ASAP until analysis. These samples were analysed for a range of herbicides including diuron, atrazine, ametryn, simazine, tebuthiuron, fluometuron, hexazinone and prometryn at QHSS.

Solvent (acetone/hexane) was added to the sediment and shaken overnight on a mechanical shaker to allow for physical separation of bulk water. The extract was then filtered through anhydrous sodium sulphate to remove residual water. This extract was then concentrated by rotary/vacuum evaporation and the solvent was exchanged to methanol/water for LC/MS/MS determination against prepared standards of the herbicides requested. A separate sediment sub-sample was used to determine dry weight for calculation. The results were expressed in micrograms per kilogram (µg/kg) on a dry mass basis.

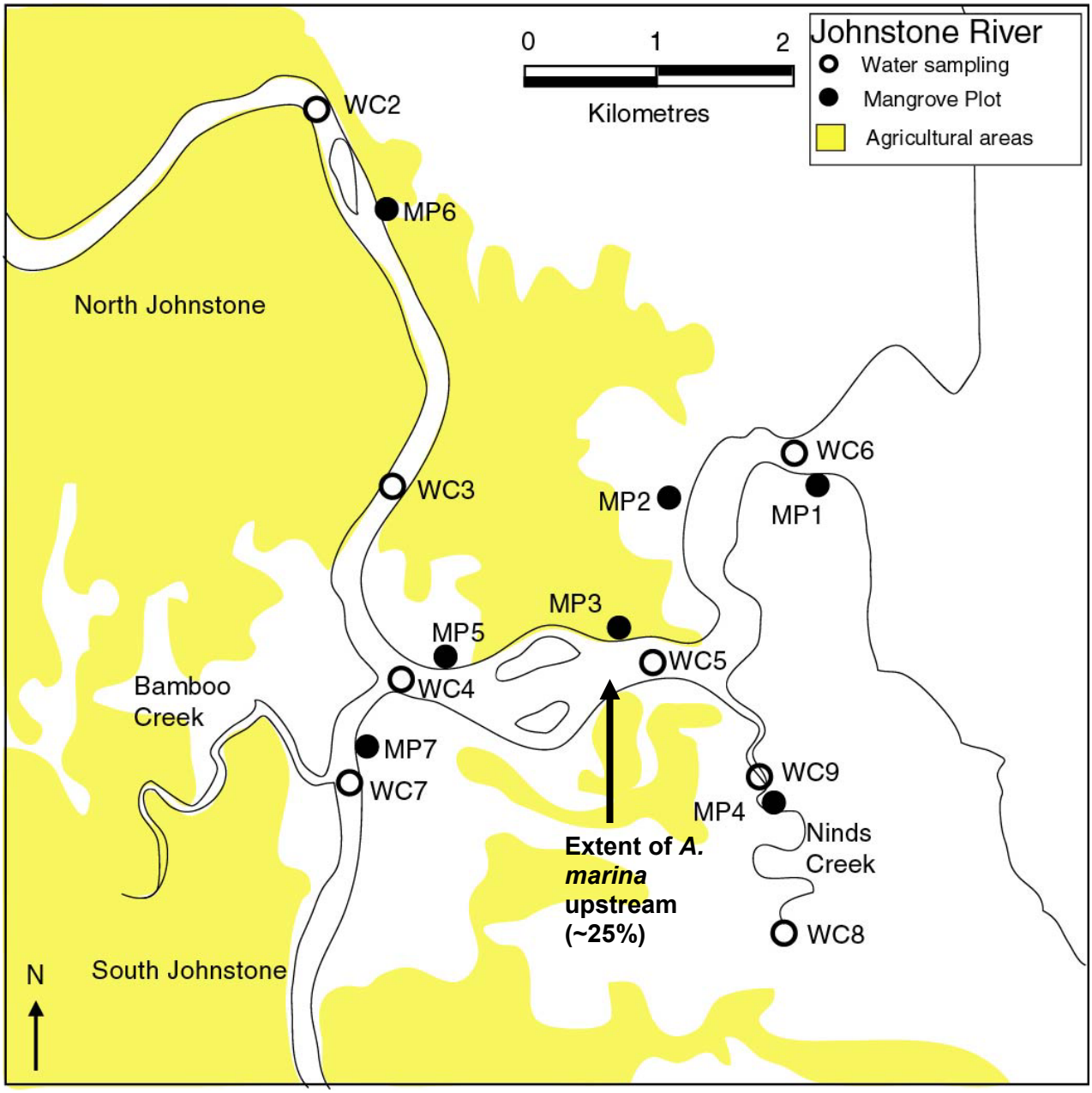

FIGURE 69: Johnstone River mangrove and water sampling sites showing extent of *A. marina* upstream (arrow) from the river mouth (for location see Figure 1).

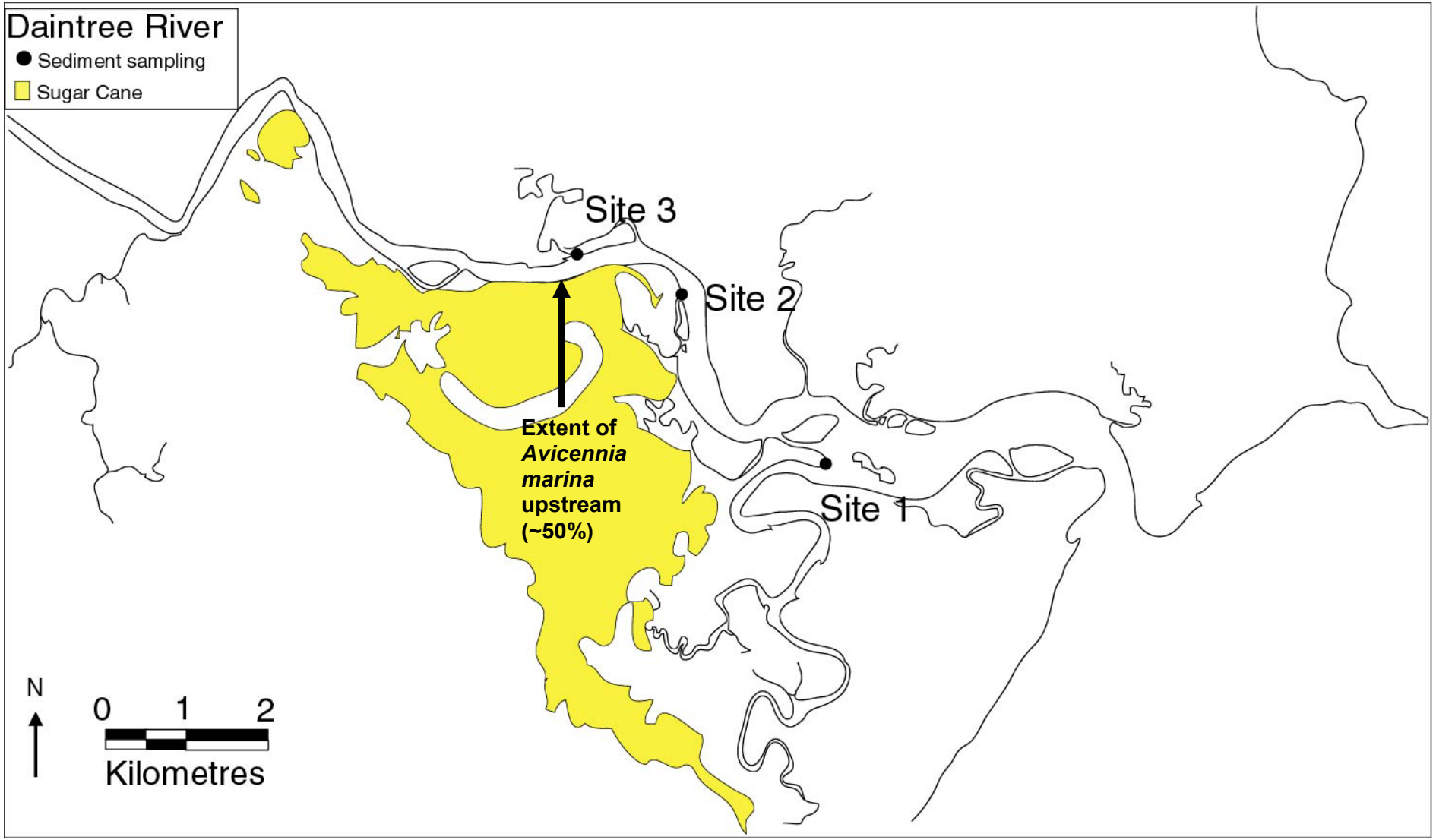

FIGURE 70: Daintree River mangrove sediment sampling sites and areas of sugar cane farming showing extent of *A. marina upstream* (arrow) from the river mouth (for location see Figure 1).

Results – Johnstone River

Mangrove Composition and Condition

All mangrove species observed in this investigation were healthy. Species found in the mangrove plots of the Johnstone River included *A. marina, A. corniculatum, Rhizophora apiculata, Bruguiera parviflora, Xylocarpus granatum, Bruguiera gymnorrhiza, Ceriops decandra, Rhizophora mucronata, Sonneratia caseolaris, Heritiera littoralis, Bruguiera sexangula, Acanthus ilicifolius* and *Cynometra iripa*. Healthy *A. marina* trees were only found in the mangrove plot MP1, but were also seen as emergent trees in the vicinity of plot MP3.

There were no *A. marina* seedlings present in any of the plots. Seedlings that were present were *A. corniculatum*, *Acanthus ilicifolius*, *Bruguiera gymnorrhiza*, *Bruguiera parviflora*, *Bruguiera sexangula* and *Xylocarpus granatum*.

Water Quality

Table 25 presents water quality data (salinity, temperature, pH and secchi depth) at the water sampling sites. Salinity was low at the upstream water sampling sites, and increased towards the mouth of the river, however remained much lower than the salinity of seawater (30 – 35 ‰). pH levels were within the ANZECC (2000) guideline levels at most water sampling sites (7.0 – 8.5 for estuaries) and were just below these levels at 3 sampling sites (ANZECC, 2000). Chlorophyll *a* values exceeded the ANZECC (2000) guidelines (0.002 mg/L) for estuaries in tropical Australia at all of the water sampling stations in the Johnstone River.

TABLE 25: Water quality parameters (salinity, temperature, pH and secchi depth) for the water sampling stations in the Johnstone River. For location of sites, refer to Figure 69.

Site Code	Location	Salinity (‰)	Temperature (^{o}C)	pH	Chlorophyll a (mg/L)	Secchi depth (m)
WC1	Nth. Johnstone	0	27.9	6.92	0.92	1+
WC2	Nth. Johnstone	2	30.3	6.67	2.15	1.5
WC3	Nth. Johnstone	5	30.7	7.13	2.36	0.83
WC7	Sth. Johnstone	15	25	7.87	2.31	1
WC4	Main River	5	30.2	7.22	1.94	0.9
WC5	Main River	10	30.4	7.3	1.71	1.05
WC6	Main River	13	30.7	7.87	2.02	1.13
WC8	Ninds Creek	15	29.9	6.89	1.61	1.1
WC9	Ninds Creek	15	30.2	7.35	1.03	0.94

Nutrients

Results for nutrients in mangrove corewater are presented in Table 26. ANZECC (2000) guidelines for nutrients in tropical Australian estuaries for slightly disturbed ecosystems (Appendix 16) were exceeded for total P at all sites, FRP at sites MP2 and MP5, ammonia at all sites, oxides of N at MP1, MP2 and MP7, and total N at all sites. Total N and Total P were highest in plot MP1 and MP5. There was no trend for increasing nutrient levels in upstream or downstream locations.

TABLE 26: Nitrogen and phosphorus concentration of mangrove core water from mangrove plots in the Johnstone River. For location of sites, refer to Figure 69.

	Phosphorus (mg/L)			Nitrogen (mg/L)			
Site Code	**Total P**	**Total Diss. P**	**Filt. Reac. P**	**Ammonia (NH_3)**	**Oxides (NO_2 + NO_3)**	**Total Diss.**	**Total N**
MP1	6.8	0.009	<0.002	0.092	0.033	0.91	23
MP2	0.06	0.014	0.007	0.009	0.087	0.29	0.49
MP5	1.4	0.038	0.013	0.12	0.007	2.5	2.9
MP6	0.65	0.011	0.003	0.29	0.008	0.44	1.1
MP7	0.55	0.022	0.002	0.039	0.2	0.42	1.3
	0.002	0.002	0.002	0.002	0.002	0.02	0.02

Heavy Metals

Results for heavy metals are presented in Table 27. ANZECC (2000) guidelines for metals and metalloids in sediments (Appendix 16) show that Chromium (Cr) exceeded the low trigger value (80 mg/kg) for sediments in all plots except plot MP1.

Nickel (Ni) exceeded the low trigger value (21 mg/kg) in all plots and the high value at sites MP3, MP6 and MP7.

Copper (Cu), Iron (Fe), Lead (Pb) and Zinc (Zn) showed trends for higher concentrations in the mangrove sediments from upstream locations than in the downstream plots.

TABLE 27: Concentrations of heavy metals (mg/kg) in surface sediments (0-2cm) of mangrove plots in the Johnstone River. For location of sites, refer to Figure 69.

SEDIMENT	**Detected elements (mg/kg)**									
Site Code	**As**	**Cr**	**Cu**	**Fe**	**Mn**	**Ni**	**Pb**	**Zn**	**Cd**	**Hg**
MP1	15	69	33	37900	540	36	10	61	<1	<0.2
MP2	9	100	37	37200	190	47	11	71	<1	<0.2
MP3	10	99	46	48300	560	56	10	74	<1	<0.2
MP4	12	86	42	43700	260	45	14	75	<1	<0.2
MP5	11	81	40	41700	250	38	12	72	<1	<0.2
MP6	13	100	53	55000	1100	56	15	84	<1	<0.2
MP7	11	110	59	59100	740	55	16	91	<1	<0.2
Detection limit	2	0.2	1	0.4	0.06	0.2	1	0.1	1	0.2

Pesticides

Sediment

The pesticides diuron, atrazine, ametryn, chlorpyrifos, arsenic and DDE were detected in the mangrove sediments in the Johnstone River (Table 28). Diuron, atrazine and ametryn are herbicides and chlorpyrifos and DDE are insecticides. Diuron was detected in the highest concentrations in the mangrove sediments and was found at all sampling sites. Higher levels of herbicides were generally detected in the plots of the upper reaches of the river, which were closer to direct agricultural influences than more downstream locations.

TABLE 28: Pesticide concentration (µg/kg dw) in the surface sediments (0-2 cm) of the Johnstone River mangrove plots. For location of sites, refer to Figure 69. ND indicates that there was no pesticide detected.

Herbicides in Sediment	**Detected Pesticides (µg/kg dw)**				
Site code	**Diuron**	**Atrazine**	**Ametryn**	**Chlorpyrifos**	**DDE**
MP1	1	ND	ND	ND	ND
MP2	0.4	ND	ND	ND	ND
MP3	0.7	ND	ND	0.1	0.2
MP4	2.6	ND	0.06	0.2	0.3
MP5	2.6	0.06	0.07	0.2	0.2
MP6	5	ND	0.02	0.5	0.2
MP7	5.2	0.11	ND	0.7	0.3
Detection limit	**0.1**	**0.1**	**0.05**	**0.1**	**0.1**

Vegetation

Results for herbicides in mangrove leaves are presented in Table 29. Diuron was detected in leaves of *A. corniculatum* and atrazine was detected in leaves of *Bruguiera sexangula* and *A. corniculatum*. The plots where herbicides were detected in mangroves leaves were situated in upstream locations (refer to Figure 69).

TABLE 29: Herbicide concentration (µg/kg) in the leaves of various mangrove species in the Johnstone River. For location of sites, refer to Figure 69. ND indicates that no herbicides were detected.

Site code	**Species**	**Diuron**	**Atrazine**
MP1	*A. corniculatum*	ND	ND
	A. marina	ND	ND
MP2	*A. corniculatum*	ND	ND
	B. parviflora	ND	ND
MP5	*A. corniculatum*	ND	ND
	B. parviflora	ND	ND
MP6	*A. corniculatum*	3.9	ND
	B. sexangula	ND	ND
MP7	*A. corniculatum*	ND	0.7
	B. sexangula	ND	1.0
Detection limit		**1.0**	**1.0**

ND indicated that there was no pesticide detected.

Results – Daintree River

Diuron was the only herbicide detected in these samples (Table 30). Concentrations of diuron in the sediments were highest in the areas closest to sugar cane farming areas (Figure 69).

TABLE 30: Concentrations of diuron, atrazine, ametryn, simazine, tebuthiuron, fluometuron, hexazinone and prometryn (µg ai/kg dw) detected in the mangrove surface sediments (0-2cm) of the Daintree River. For location of sites, refer to Figure 70. ND indicates that no herbicides were detected.

Site	Location	Diuron	Atrazine	Ametryn	Simazine	Tebuthiuron	Fluometuron	Hexazinone	Prometryn
Site 1	Downstream	0.1	ND	ND	ND	ND	ND	ND	ND
Site 2		1.1	ND	ND	ND	ND	ND	ND	ND
Site 3	Upstream	0.64	ND	ND	ND	ND	ND	ND	ND
Detection limit		0.1	0.1	0.05	0.1	0.1	0.1	0.1	0.1

Discussion Comparing Rivers

This study was needed to answer the question of why mangrove dieback was apparently only occurring in Mackay and not other river systems. Possible causes for dieback (heavy metals, nutrients and pesticides) were compared between healthy *A. marina* plots in the Johnstone River and in dieback areas in Mackay.

Concentrations of the metals As, Cr, Cu, Fe, Mn, Ni, Pb, and Zn In the Johnstone River were either in the same range or much higher than they were in the Mackay sediments. At site MP1, where healthy *A. marina* trees existed, the levels of As, Cr, Fe, Mn, and Zn exceeded the highest levels of these metals in the Mackay sediments of the dieback areas. From this, it can be concluded that metals were highly unlikely to be causing the *A. marina* dieback in Mackay. Ni and Cr exceeded ANZECC (2000) guidelines at a number of sites, however, these guidelines are normalised to 1% organic carbon, so if the sediment organic carbon content is markedly higher than 1%, the guideline value should be made less stringent because additional carbon binding sites reduce bioavailablility of contaminants (ANZECC, 2000). Given that other % organic carbon measured in mangroves in Mackay averaged at about 10%, these levels of heavy metals in the sediment may not be harmful.

Nutrient levels as indicated by N and P concentrations in mangrove corewater and by chlorophyll *a* in the water column, were also very high in the Johnstone River. In the plot where healthy *A. marina* was found (MP1), the levels of N and P greatly exceeded the concentrations found in the Mackay core water. Furthermore, ammonia in the core waters of the plots with healthy *A. marina* in the Johnstone River exceeded ammonia concentrations in all Barnes Creek and McCreadys Creek plots. No pH measurements were taken of mangrove corewater to determine whether ammonia could pose a problem for these trees.

As with the estuaries surveyed in Mackay, the levels of chlorophyll *a* in the Johnstone River exceeded the guideline levels in the ANZECC (2000) water quality guidelines. Chlorophyll *a* levels were variable in the river but lowest at the farthest upstream and downstream locations. Nutrient inputs are from non-point sources such as runoff from agricultural area, but also point sources, such as the Innisfail Wastewater Treatment plant located on the western bank of Ninds Creek and the meatworks in Innisfail.

The herbicides diuron and atrazine were detected in mangrove leaves in upstream plots, demonstrating that in a field situation, mangroves do take up herbicides. Presumably if a herbicide is in the leaves of a plant, it is not good for the plant. It is interesting to note that both a salt-excreting species, *A. corniculatum*, and a salt-excluding species, *Bruguiera sexangula*, had similar detectable amounts of herbicides in the leaves in plot MP7. The sites with *A. marina* also coincided with the lowest concentrations of diuron found in the mangrove plot surface sediments, while plots with comparatively higher levels did not contain any *A. marina* in or nearby them. Levels of diuron in the Daintree River were also quite low where there was healthy *A. marina*. Concentrations of diuron where *A. marina* were present, both in the Johnstone and the Daintree Rivers did not exceed 1.1 µg/kg dw in the mangrove sediment – See Figure 71. The lowest concentration of diuron found in Mackay in the present study, was 1.0 µg/kg at site M2, where mangroves appeared to be resprouting leaves from epicormic buds under the bark.

It may be purely coincidental that *A. marina* was only present in plots where herbicide concentrations were low. It could be that *A. marina* was never present in these upstream locations to begin with, or that their appearance at the mouth of the river was due to other factors such as water salinity, regardless of the presence or absence of toxic substances in the sediment. There was also the possibility that *A. marina* once existed further upstream in the Johnstone River, but since the commencement of intensive farming practices a number of decades ago, *A. marina* have been replaced by more herbicide-tolerant mangrove species.

To provide an answer to this question, it is important to obtain historical records (such as aerial photographs) of mangrove composition, distribution and condition. It is also important to compare river systems with similar attributes (rainfall, temperature and anthropogenic influences) to determine whether such a species distribution is common elsewhere. Table 31 presents information on *A. marina* distribution in a number of Northern Queensland estuaries with comparable conditions. From this table, it can be seen that in the Johnstone River, *A. marina* occupies only ~25% of the lower estuary, while the vast majority of other river systems with lower agricultural inputs such as the Daintree River have a greater upstream distribution of *A. marina.* Clearly, further data collection and investigation must be carried out before any conclusions can be made.

TABLE 31: *A. marina* distribution in comparable North Queensland river systems relative to the herbicide usage in each catchment (Hamilton and Haydon, 1996; NC Duke, unpublished survey data).

River System	*A. marina* as % of estuary	*A. marina* distance upstream (km)	*A. marina* distance downstream (km)	Estuary Length (km)	Annual Rainfall (mm)	Annual app. diuron
Jackie Jackie	100	31.0	6.2	31.0	1800	no data
Escape	50	10.0	3.0	20.0	1800	no data
Harmer	62	2.5	1.5	4.0	1600	no data
Macmillan	100	5.0	0.0	5.0	1700	no data
Oliver	62	15.0	-	24.0	1600	no data
Pascoe	70	7.7	0.4	11.0	1500	no data
Claudie	90	8.4	1.9	9.0	1500	no data
Lockhart	70	12.0	3.3	17.0	1400	no data
Nesbit	100	4.0	-	4.0	1400	no data
Rocky	100	2.0	0.0	2.0	1400	no data
Normanby	80	39.0	0.5	48.5	1300	no data
Annie	100	22.0	5.0	22.0	1200	no data
Jeanie	100	6.0	1.0	6.0	1400	no data
McIvor/Morgin	100	10.0	0.0	10.0	1700	no data
Endeavour	61	11.0	1.5	18.0	1800	no data
Bloomfield	19	1.5	0.5	8.0	2000	no data
Daintree	50	10.5	5.0	20.0	2086	2378
Johnstone	25	3.45	0.0	13.8	3664	17353
Pioneer	100	8.0	0.5	8.0	1385	23435

Regardless of whether or not *A. marina* had previously existed in the upper reaches of the Johnstone River, it is clear that there are a number of issues that need to be addressed or revised for both the Johnstone and Daintree Rivers. One of the most important issues is that herbicides are not naturally found in the environment, and they should not be in the marine environment. Presumably if high levels of herbicides and nutrients are detected at the mouth of the Johnstone River, then they may have reached offshore marine environments such as seagrass and coral, which have been observed to be sensitive to such inputs (eg. Haynes *et al.,* 2000b; Jones *et al.,* 2003).

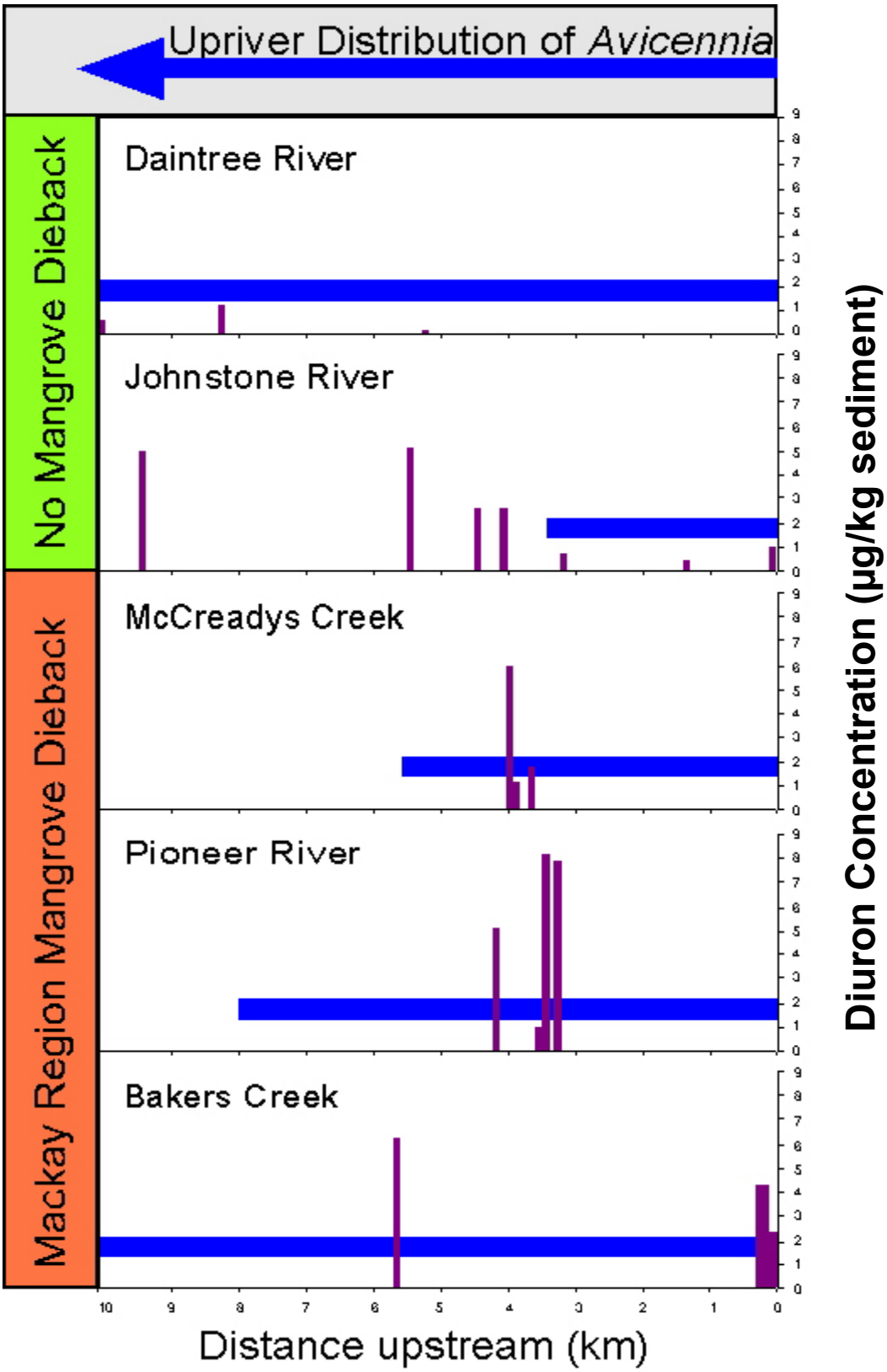

FIGURE 71: Herbicide concentrations (µg/kg) in surface sediments (0-5cm) of mangrove plots in the Daintree River, Johnstone River, McCreadys Creek, Pioneer River and Bakers Creek and the upriver distribution of *A. marina*

FIGURE 72: (A) Taking sediment samples in the Johnstone River mangroves (B) Sampling water quality in the Johnstone River (C) Measuring plot demography and composition in the Johnstone River mangroves (D) Healthy *A. marina* in the Daintree River.

Overall Discussion and Conclusions

Investigations undertaken in 2002 were based on 3 research components chosen to complement and progress our overall enquiry, and to identify the cause of dieback.

1) Surveys of Mangrove Dieback in the Mackay Region

- Aerial and field surveys to describe and map the extent of dieback, and to compare plant condition with measurements of the most likely agents causing dieback, including sediment burial, excess heavy metals, excess nutrients and herbicides.

2) Preliminary Toxicology Trials in the Planthouse

- Planthouse trials to show effects of 3 herbicides (deemed the most likely agents for causing dieback) on 4 mangrove species, comparing differences between salt excluding and salt excreting species.

3) Comparative River Studies Assessing *A. marina* and Herbicide Presence

- Field surveys of other river systems to compare mangrove conditions and herbicide (the most likely agents for causing dieback) with those in the Mackay.

These components address a selection of key questions raised in the preliminary investigation (Duke *et al.,* 2001). The questions are answered briefly based on the findings of the 2002 investigations reported here. Our specific recommendations based on these findings are presented in the section on Key Findings and Recommendations.

1) Surveys of Mangrove Dieback in the Mackay Region

Was the total area of dieback expanding? **Not surveyed.**
Was it possible to establish a baseline record of dieback in 2002? **Yes.**

- A baseline for Pioneer River, Bakers Creek and McCreadys Creek was established for future assessments with interpretative maps drawn from aerial photography taken in September 2002 (see Figures 11 to 18).

- The full extent of dieback in the region remains unknown (see Duke *et al.,* 2001 and this report).

What was the current extent of mangrove dieback in individual estuaries, and in the region?
Dieback mapped for key estuaries in the Mackay region.

- Widespread severe, species-specific dieback of mangroves based on the common mangrove species, *A. marina*, was observed in 5 estuaries in the Mackay region from Sandringham Bay to Reliance and Leila Creeks (see Figures 2 and 3).

- There was no dieback of *A. marina* in Eimeo/ Bucasia Creeks, however the abundance of *A. marina* in these creeks was very low compared with other estuaries mapped in this investigation (see Figures 11 and 12).

- The total area affected by dieback in McCreadys Creek was around 34 ha, ~8% of mangrove areas. Approximately 91% of dieback areas involved *A. marina*. The other type of dieback was dominated by *Ceriops australis* (Table 5).

- The total area affected by dieback in Pioneer River estuary was around 346 ha, ~55% of mangrove areas, and this included most mangrove communities with *A. marina* (see Table 5).

- All mangrove areas were affected by severe and moderate dieback in Pioneer River estuary in September 2002, including Basset Basin, Barnes Creek, Vines Creek and Fursden Creek, (see Figures 9 and 16).

- 173 hectares of *A. marina* mangroves in Bakers Creek were affected by moderate and severe dieback, which was approximately 40% of the total area of mangroves in the area. Dieback affected less than 1 hectare of mangrove species other than *A. marina* in Bakers Creek (see Table 5).

- Mangrove dieback in the vicinity of agricultural drains was severe, noting condition was marked by either greater portions of dead and unhealthy trees of *A. marina*, their absence, or as apparent growth deformities of pneumatophores (see Figures 16 and 18, Figure 57).

***Was* A. marina *dieback the more dominant kind of dieback?* Yes.**

- *A. marina* dieback dominated all other kinds of mangrove dieback in the Mackay region, notably in estuaries of the Pioneer River, McCreadys Creek and Bakers Creek (see Figures 11 to 18, Table 5).

- Most mangrove dieback areas involved *A. marina* as either a dominant or minor component of total mangrove forest composition (see Table 5).

***Was dieback getting worse?* Yes.**

- Dieback was worse where mangrove health deteriorated further since 2000, and the proportion of healthy trees declined further in plots re-sampled in 2002, Barnes Creek, Pioneer River (see Figure 46).

- Mangrove dieback commenced possibly in the early to mid 1990's in the Pioneer River notably seen in aerial photographs of upstream areas around Fursden Creek (see Appendices 6 to 8).

***Was mangrove (notably* A. marina*) health and condition correlated with sediment burial, excess heavy metals, or excess nutrients?* No.**
***What was the relationship between pneumatophore height and mangrove health for* A. marina*?* No relationship.**
***Had root burial selectively effected* A. marina *trees and contributed to their death?* No.**

- Mangrove canopy and tree health were not correlated with other key factors likely to cause dieback including sediment burial (indicated by pneumatophore height of *A. marina*, usually ~5-15 cm), heavy metals (including, Pb, Hg, Mn, Cu, Cd), or nutrients (including N and P) (see Tables 9, 10 and 11).

***Was mangrove (notably* A. marina*) health and condition correlated with herbicide (notably diuron) concentrations?* Yes.**
***Was there seedling recruitment and recovery?* No.**

- Mangrove mature canopy health correlated with diuron in sediments where higher diuron concentrations were found in sediments with fewer healthy trees of *A. marina*, measured as leaf chlorophyll concentrations (see Figures 52 and 53).

- Mangrove seedling health correlated with diuron in sediments where higher diuron concentrations found in sediments with fewer healthy seedlings of *A. marina*, measured as % healthy seedlings (see Figure 54).

Were herbicide residues similar in adjacent estuaries in the Mackay region? **Yes.**

- Unusually high residue concentrations of herbicide diuron were detected in mangrove sediments (~6-8 µg/kg) and core water (~8-14 ng/L) of 3 estuaries in the Mackay region, McCreadys Creek, Pioneer River and Bakers Creek (see Figures 48 and 51, Table 19).

Were herbicide residues higher in upstream water? **Yes.**
What was the condition of **A. marina** ***in agricultural drains, and what herbicides were present?*** **Poor health and diuron present.**

- Herbicide concentrations were highest at upstream water sites, particularly in samples collected from agricultural drains entering mangrove areas of Pioneer River (up to 1,151 ng/L, diuron) and Bakers Creek (up to 534 ng/L, diuron) (see Table 15, Figure 71).

Had there been a change in herbicide concentrations found in plots sampled in 2000? **The same.**

- Herbicide concentrations were unchanged from 2000, noting diuron concentrations were the same in mangrove sediments re-sampled in 2002 in Barnes Creek, Pioneer River (see Figures 49 and 50).

What were the consequences of the dieback? **Erosion observed.**

- Erosion and deposition were apparently accelerating in dieback areas, noted as erosion from higher intertidal areas and deposition into lower intertidal areas including stream channels (see Figure 56).

2) Preliminary Toxicology Trials in the Planthouse

Were mangroves affected by herbicides? **Yes.**
If so, was **A. marina** ***more sensitive than other species commonly found in Mackay region?*** **Yes.**
Were salt excreting species more sensitive than salt excluding species? **Yes.**

- Mangroves were affected by herbicides in sediments and water, noting affects on seedlings: leaf chlorosis, necrosis and premature abscission; loss of photosynthetic function; wilting; and death (see Figures 61 to 64, and 68).

- *A. marina* was more sensitive to herbicides than other mangrove species tested, with salt-excreter species (*A. marina* > *A. corniculatum)* affected more by herbicides than salt excluders *(R. stylosa* > *C. australis)* (see Figures 61 to 64).

Was diuron worse than other herbicides found in mangrove sediments? **Yes.**

- Diuron was the most toxic herbicide tested, and herbicides were ranked by toxicity to mangrove seedlings from most to least toxic: diuron > ametryn > atrazine. Due in large part to the more rapid breakdown of atrazine (see Figures 64 and 66).

3) Comparative River Studies Assessing *A. marina* and Herbicide Presence

Were mangroves affected by herbicides in other rivers? **Not in 2002, but possibly earlier.**

- There were no observations of mangrove dieback associated with herbicides outside the Mackay region in 2002 (this report, see Chapter 3).

Had species-specific dieback only occurred in the Mackay region? **Further study needed.**

- The only other observation of *A. marina* dieback was from the Gladstone region in the early 1970s. The cause of this incidence of dieback was never discovered. It was suggested that plant pathogens were involved but their presence is currently believed to be a consequence of dieback rather than the cause (see Duke *et al.,* 2001; Prof John Irwin, UQ, pers. com).

- There have been no comparable surveys of mangrove health in other estuaries, and incidents of severe mangrove dieback have not been recorded in any state or national database.

Was* A. marina *found in other river systems where herbicide levels were as high as those found in Mackay region? **Yes, but they were mutually exclusive in occurrence.**

- Johnstone and Daintree River mangrove sediments had comparable herbicide levels, however, *A. marina* was absent where diuron concentrations were >2 µg/kg. Areas of *A. marina* occurrence and dieback in Mackay region had herbicide concentrations 6-8 times higher than *A. marina* areas in Johnstone and Daintree Rivers. *A. marina* trees were healthy in these river estuaries in 2002 (see Figure 71).

Why was the dieback only occurring around Mackay in recent years? **Increased area of planted cane and high usage of herbicides in catchments.**

- Recent serious dieback might be the result of increased use of herbicides in the catchment over the last 10 years (see Table 20, Duke *et al.,* 2001).

- Dieback may have occurred elsewhere and gone unnoticed in estuaries where *A. marina* was less common, like the Johnstone River estuary (see Figure 71; Table 31).

Conclusion

These findings provide strong correlative and some causative evidence implicating herbicides, particularly diuron, as the primary agent causing serious dieback of mangrove in the Mackay region. The key facts appear inescapable. The health of mangroves in the region were in serious decline in 2002, and they had gotten worse over the previous 2 years. This presents serious implications for associated marine habitats and coastal areas irrespective of the cause. There was also no doubt that significant loads of herbicides were washed downstream in runoff from cane agricultural areas each year. There was also no doubt that herbicide concentrations in mangrove sediments remained at unusual and relatively high levels. As to the cause, it perhaps should not be surprising that an agent (a herbicide) manufactured to kill plants via root uptake might affect a non-target species like *Avicennia marina*, the affected mangrove in estuaries downstream from the agricultural areas.

As noted, the dieback appeared unprecedented but it could have already occurred elsewhere. It is of serious concern that declines in this vulnerable species might have gone unnoticed in other high use areas. It is also of concern that more cryptic plant marine species like some seagrasses and

corals might also have died off without being noticed. The implications of these hidden and unknown responses might be considerable and far reaching. For example, it is certainly not unreasonable to ask what might be the effects of such run-off on coral growth and survival on the Great Barrier Reef.

During this investigation, we have taken great care to consider all possible causes of the severe dieback, and finally targeting the more likely causes including sediment deposition, high nutrients and toxic chemicals. Our current results are fully presented in this report. The report has already undergone rigorous scientific review by at least five independent specialists in the field. We have addressed all their comments, criticisms and queries, and incorporated their comments and our responses into this final version.

We accept that our conclusions are dependant on data currently available. Nevertheless, based on these findings and data, we believe there is compelling and sufficient evidence to identify herbicides as the agent responsible for the serious dieback of mangrove in the Mackay region. By contrast, there has been no contradictory data or other substantive evidence to suggest any other primary causative agent. We therefore recommend that immediate action be taken to reduce the amount of harmful herbicides reaching affected mangrove areas as the best way to halt the decline in mangrove dieback. Only once the concentrations of herbicides in sediments have been reduced (below 2 µg/kg), can we expect to see an improvement in overall mangrove plant recovery and recruitment required to restore these seriously damaged estuarine habitats.

We provide specific recommendations to assist in subsequent investigations and monitoring.

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FIGURE 32: Bakers Creek transect 2 (BCT2) was run from mid-mangrove to the terrestrial margin (T) of the mangroves. Condition (healthy, sick or dead) and proportion of *A. marina* was represented in plots along the transect. *A. marina* was classified as having none (N), moderate (M) or severe (S) dieback based upon their condition in the plot. A sample for herbicide analysis (star) was taken from the mangrove core water (Table 15). For location of the transect, refer to Figure 6.

FIGURE 33: Photosynthetic activity of *A. marina* trees. Moderate plots had 15 –30 % and severe plots had >30% of *A. marina* trees as sick or dead. Refer to Figures 4, 5 and 6 for site locations. Error bars are standard error terms for replicated measurements.

FIGURE 34: Chlorophyll *a* concentrations in *A. marina* mangrove leaves from McCreadys Creek, Barnes Creek and Bakers Creek. Error bars are standard error terms for replicated samples.

FIGURE 35: Percentage organic composition of the mangrove sediment in McCreadys Creek, Bakers Creek and Barnes Creek. Error bars are standard error terms for replicated samples.

FIGURE 36: (A) Total chlorophyll in sites throughout the Mackay region **(B)** % unhealthy (sick) and dead *A. marina* trees in mangrove plots. Refer to Figures 4,5 and 6 for site location.

FIGURE 37: Significant correlation (r^2 =0.7525, n =28, P<0.001) between total nitrogen and total phosphorus in the core water from all plots in the Mackay region (including data from Duke *et al.,* 2001). Data was log transformed.

FIGURE 38: Significant correlation (r^2=0.6467, n=11, p<0.002) between the % nitrogen in *A. marina* leaves and total nitrogen in the core water.

FIGURE 39: Significant correlation (r^2=0.3513, n-11, p<0.05) between the % nitrogen in *A. marina* leaves and the concentration of ammonia (mg/L) in the mangrove sediment core water.

FIGURE 40: Carbon 13 in *A. marina* from healthy and unhealthy (sick) leaves. Leaves were taken from trees in McCreadys Creek, Barnes Creek and Bakers Creek.

FIGURE 41: Chlorophyll *a*, *b*, *c* and carotenoid concentrations in McCreadys Creek, with ANZECC guideline (2000) levels for chlorophyll *a* for slightly disturbed ecosystems in tropical Australia. Sites are located from upstream to downstream. Refer to Figure 4 for details of locations.

FIGURE 42: Chlorophyll *a*, *b*, *c* and carotenoid concentrations in the Pioneer River, with ANZECC guideline (2000) levels for chlorophyll *a* for slightly disturbed ecosystems in tropical Australia. Sites are located from upstream to downstream. Refer to Figure 5 for details of locations.

FIGURE 43: Chlorophyll *a*, *b*, *c* and carotenoid concentrations in Bakers Creek, with ANZECC guideline (2000) levels for chlorophyll *a* for slightly disturbed ecosystems in tropical Australia. Sites are located from upstream to downstream. Refer to Figure 6 for details of locations.

FIGURE 44: Pneumatophore heights above the sediment surface and distance below ground to cable roots of *A. marina* for each of the mangrove plots in the 3 estuaries. For location of mangrove plots, refer to Figures 4, 5 and 6. Error bars are standard error terms for replicated samples.

FIGURE 45: Relationship between the average % algal inhibition in the Toxy-PAM and the sediment core water and river water diuron concentration (r^2=0.3655, $p<0.05$, n=10) from samples in the Mackay dieback areas.

FIGURE 46: Mangrove plots M1 (A) and M2 (B) comparing the health of *A. marina* in 2000 (Duke *et al.,* 2001) and 2002 (current investigation).

FIGURE 47: Mouth of Bassett Creek, draining into the Bassett Basin (Pioneer River Estuary) with freckled patches of sick and dead *A. marina* amongst other live species. Photos by Mr. Serge de Pinto (25 May, 1998).

FIGURE 48: Average diuron concentration from the study sites in McCreadys Creek, Barnes Creek and Bakers Creek, comparing concentrations in the sediment (µg/kg) and the mangrove plot core water (ng/L).

FIGURE 49: Comparison in diuron concentrations between site M1 and M2 over two sampling periods (2000 and 2002). Average diuron concentration and the range of diuron found in these two sites are provided for reference. The detection limit for diuron is as indicated (0.1µg/kg).

FIGURE 50: Comparison in ametryn concentrations between site M1 and M2 over two sampling periods (2000 and 2002). Average ametryn concentration and the range of ametryn found in these two sites are provided for reference. The detection limit for diuron is as indicated (0.05 µg/kg).

FIGURE 51: Relationship (r^2=0.9855, n=3, $p<0.01$) between the average concentration of diuron in the mangrove sediments in the Mackay region and the % area of healthy *A. marina* trees, as quantified by the mapping exercise.

FIGURE 52: Relationship between healthy *A. marina* trees in the plot and diuron concentration in the mangrove sediment (0-5 cm depth) for Barnes Creek (r^2=0.892 $p<0.02$, n=4) Bakers Creek (r^2=0.8179, $p<0.05$, n=4) McCreadys Creek (no relationship) and the overall relationship (r^2=0.4803, $p<0.02$, n=11).

FIGURE 53: Relationship between average chlorophyll *a* concentration (from healthy and sick leaves) in *A. marina* leaves and total herbicide concentration in the sediments for Barnes Creek (r^2=0.8995, $p<0.02$, n=4) Bakers Creek (r^2=0.9915, $p<0.005$, n=3) and McCreadys Creek (no relationship).

FIGURE 54: Relationship between herbicide concentration in the sediment and % healthy *A. marina* seedlings in each plot in Barnes Creek (r^2=0.8069, n=4, p<0.05), Bakers Creek (r^2=0.9524, n=3, p<0.05), McCreadys Creek (r^2 = 0.9995, n=3, p<0.005) and overall correlation (r2=0.3352, n=11, p<0.05). Healthy seedlings included seedlings with insect attack, as sick seedlings were counted as those with yellow leaves.

FIGURE 55: (A) Herbicide movement into the marine environment and the effects of herbicides on the biota. **(B)** Sediment shifting in the mangrove zone, with erosion evident in higher tidal parts where there are dead trees, and with deposition in lower tidal parts along creek margins.

FIGURE 56: (A) An erosion gully in Barnes Creek in 2000 **(B)** The same erosion gully in 2002 where the gully has widened and trees have fallen over. Salt marsh is moving into light gaps created by the dieback. **(C)** Major sheet erosion in the upper intertidal areas showing the exposed roots of *A. corniculatum* **(D)** A scree slope in Barnes Creek with newly established seedlings **(E)** Resprouting from epicormic buds in *A. marina* **(F)** Severe gully erosion in Barnes Creek mangroves.

FIGURE 57: (A) Healthy *A. marina* next to Bakers Creek meatworks output. Note bubbly water (Photo Judith Wake) **(B)** Bakers Creek, with sedimentation affecting *R. stylosa* **(C)** Anomalous pneumatophores of *A. marina* in drain at Bakers Creek **(D)** Drain water sampling in Bakers Creek.

Planthouse Study

FIGURE 58: Schematic diagram of the Photosystem II complex, situated within the plant thylakoids. Source: Wolfson Laboratories (2002).

FIGURE 59: Root-tip structure of a salt-excluder, *Bruguiera gymnorrhiza* and a salt-excretor, *A. marina* (Lawton *et al.,* 1981).

FIGURE 60: (A) Experimental design in the planthouse, consisting of 12 individual tidal tank systems, four treatment groups, and four concentrations of herbicides applied to four mangrove species. **(B)** Tidal tank set-up in the mangrove planthouse, showing different tidal regimes and problems associated with plant height. Taller trees (*R. stylosa* and *A. marina*) had leaves emerging above the high water mark while shorter trees (*A. corniculatum* and *C. australis*) had leaves submerged during the high tide.

FIGURE 61: (A) Maximum potential quantum yield (F_v/F_m) responses of *R. stylosa* (■), and *A. marina* (♦) with root-only exposure to diuron at the highest dosage concentration (4000 µg/kg). **(B)** Maximum potential quantum yield (F_v/F_m) responses of *C. australis* (**X**) and *A. corniculatum* (▲) with both foliage and root exposure to diuron at the highest dosage concentration (4000 µg/kg). Units of maximum potential quantum yield are arbitrary (error = 1 SEM). Numbers in brackets indicate percent mortality (n=6 for each species).

FIGURE 62: (A) Maximum potential quantum yield (F_v/F_m) responses of *R. stylosa* (■, n=6) *A. marina* (♦, n=6) and *A. corniculatum* (▲, n=1) with root-only exposure to ametryn at the highest dosage concentration, (4000 µg kg $^{-1}$). **(B)** Maximum potential quantum yield (F_v/F_m) responses of *C. australis* (**X,** n=6) and *A. corniculatum* (▲, n=5) with foliage and root exposure to ametryn at the highest dosage concentration, (4000 µg/kg). Units of maximum potential quantum yield are arbitrary (error =1 SEM). Numbers in brackets indicate percent mortality.

FIGURE 63: (A) Maximum potential quantum yield (F_v/F_m) responses of *R. stylosa* (■) and *A. marina* (♦) with root-only exposure to atrazine at the highest dosage concentration,
(4000 µg kg $^{-1}$). (B) Maximum potential quantum yield (F_v/F_m) responses of *C. australis* (X) and *A. corniculatum* (▲) with both foliage and root exposure to atrazine at the highest dosage concentration, (4000 µg/kg). Units of maximum potential quantum yield are arbitrary (error =1 SEM). Numbers in brackets indicate percent mortality (n=6 for each species).

FIGURE 64: Maximum potential quantum yield responses of *A. marina* in control (O) tanks, and with exposure to diuron (▲), atrazine (♦) and ametryn (■) at the highest dosage concentration (4000 µg/kg). Units of maximum potential quantum yield are arbitrary (error =1 SEM).

FIGURE 65: Diuron uptake in mangrove leaves (µg/kg) after 11 days of exposure to 4000 µg/kg dosage of diuron in **(A)** root-exposed plants only (*A. marina* and *R. stylosa*) **(B)** root and foliage exposed plants (*A. corniculatum* and *C. australis)* (error term = 1 SEM).

FIGURE 66: Total calculated concentrations of diuron, ametryn and atrazine (µg/kg) in sediments in the top 1cm of the sediment from pots in the planthouse experiments. Concentrations displayed are a total of all concentrations in the tanks, to calculate degradation rate. This was to normalise for accidental transfer between pots with different doses.

FIGURE 67: Del15N uptake in leaves from *A. marina*, *A. corniculatum* and *R. stylosa* from various locations in the Pioneer River, Mackay, Australia (Duke *et al*., 2001).

FIGURE 68: Planthouse studies at Moreton Bay Research Station (A) Tank set-up in the Planthouse (B) Seedlings with *A. marina* showing chlorosis (C) Chlorotic *A. marina* in the early stages (D) Chlorosis in *A. corniculatum* and wilting of leaves (E) Later stages of chlorosis in *A. marina* and beginning stages of necrosis (F) Necrosis in *A. marina.*

Comparative Study

FIGURE 69: Johnstone River mangrove and water sampling sites showing extent of *A. marina* upstream (arrow) from the river mouth (for location see Figure 1).

FIGURE 70: Daintree River mangrove sediment sampling sites and areas of sugar cane farming showing extent of *A. marina upstream* (arrow) from the river mouth (for location see Figure 1).

FIGURE 71: Herbicide concentrations (µg/kg) in surface sediments (0-5cm) of mangrove plots in the Daintree River, Johnstone River, McCreadys Creek, Pioneer River and Bakers Creek and the upriver distribution of *A. marina*

FIGURE 72: (A) Taking sediment samples in the Johnstone River mangroves **(B)** Sampling water quality in the Johnstone River **(C)** Measuring plot demography and composition in the Johnstone River mangroves **(D)** Healthy *A. marina* in the Daintree River

Appendices

APPENDIX 1: Mosaic image of Eimeo used to classify mangrove dieback in the current investigation

APPENDIX 2: Mosaic image of Bucasia Creek used to classify mangrove dieback in the current investigation

APPENDIX 3: Mosaic image of McCreadys Creek used to classify mangrove dieback in the current investigation

APPENDIX 4: Mosaic image of the Pioneer River estuary used to classify mangrove dieback in the current investigation

APPENDIX 5: Mosaic image of the Bakers Creek estuary used to classify mangrove dieback in the current investigation

APPENDIX 6: Aerial photograph of Fursden Creek in the Pioneer River estuary in 1993 (Source: Mackay Sugar).

APPENDIX 7: Aerial photograph of Fursden Creek in the Pioneer River estuary, taken on 2/11/1997. Note obvious patches in canopy (Source: Mackay Sugar).

APPENDIX 8: Aerial photograph of Fursden Creek in the Pioneer River estuary, taken on 2/09/2002.

APPENDIX 9: STP creek draining into Bassett Basin in 1993. Healthy *A. marina* trees were present (Source: Mackay Sugar).

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APPENDIX 20: Rainfall and temperature data for Mackay MO from the years 1959 to 2002 (Source: Bureau of Meteorology Australia, 2002)

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APPENDIX 1: Mosaic image of Eimeo used to classify mangrove dieback in the current investigation

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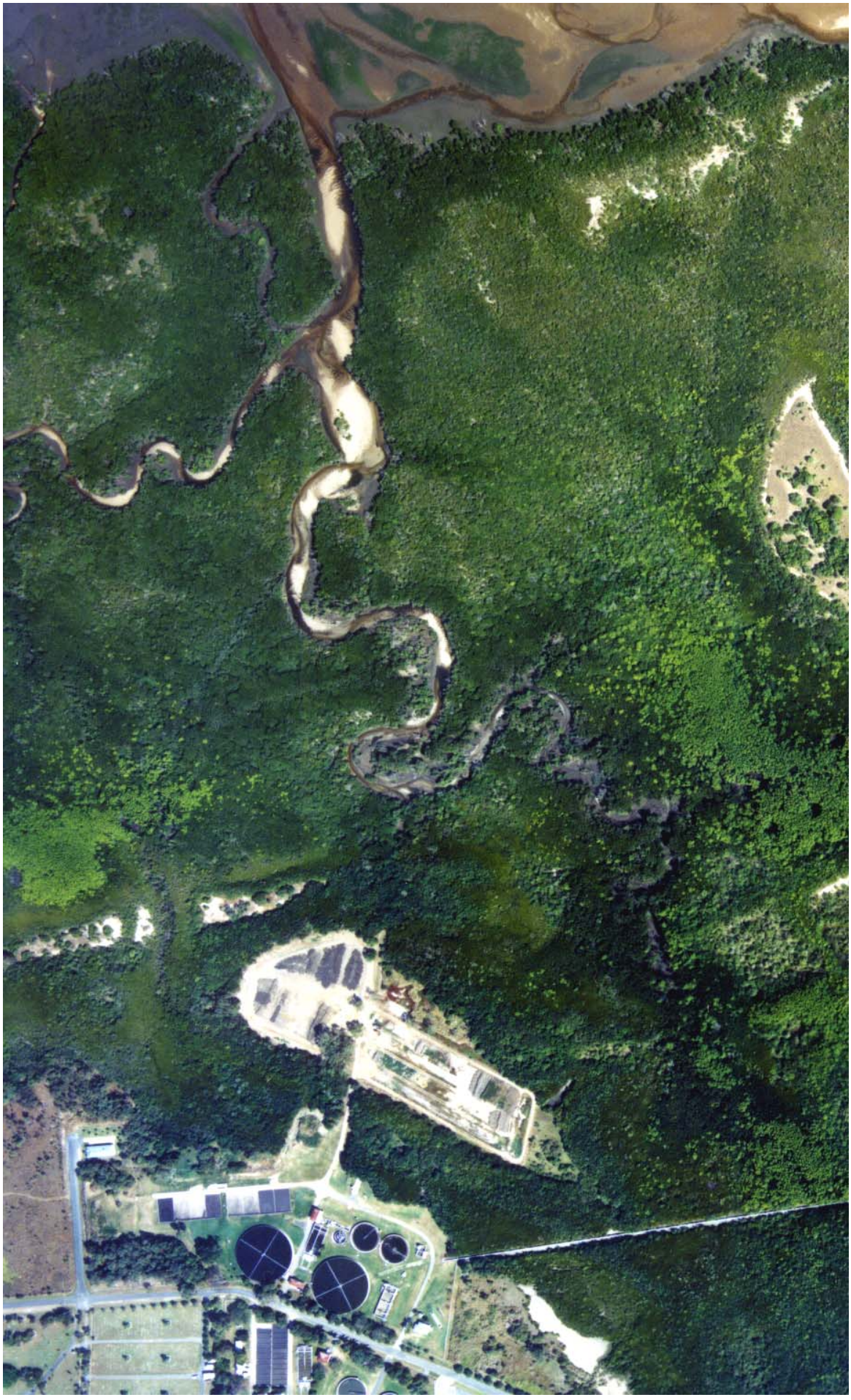


APPENDIX 10: Waste water treatment plant discharging into the 'STP creek' draining into Bassett Basin on 2/09/2002.

APPENDIX 11: Transect GPS data for McCreadys Creek, Barnes Creek (Pioneer River) and Bakers Creek, including stake heights at the start of each rapid transect.

MCCREADYS CREEK TRANSECTS

MCT1	McCreadys Creek		
Plot #	Lat/Long		Stake height
1	21.07421	149.18802	Left: 99cm, Right: 104.5cm
2	21.07425	149.18801	
3	21.07431	149.18802	
4	21.07436	149.18804	
5	21.07439	149.18801	
6	21.07443	149.18799	
7	21.07451	149.18745	
8	21.07454	149.18792	
9	21.07457	149.18789	
10	21.07464	149.18784	
11	21.07471	149.18784	
Final stake	21.07477	149.18779	

MCT2	McCreadys Creek		
Plot #	Lat/Long		Stake height
1	21.07451	149.18778	Left: 110.4cm, Right: 97.5 cm
2	21.07459	149.18775	
3	21.07463	149.18771	
4	21.07466	149.18768	
5	21.07472	149.18765	
6	21.07476	149.18764	
7	21.07481	149.18758	

BARNES CREEK TRANSECTS (PIONEER RIVER)

BT1	Barnes Creek		
Plot #	**Lat/Long**		**Stake height**
1	21.12959	149.19058	Left: 128.8 cm, Right: 130.4cm
2	21.12955	149.19063	
3	21.12955	149.19068	
4	21.12956	149.19069	
5	21.12954	149.19088	
6	21.12946	149.19072	
7	21.12941	149.19076	
8	21.1294	149.19077	
9	21.1293	149.19073	
10	21.12928	149.19077	
11	21.1293	149.1909	
BT2	**Barnes Creek**		
Plot #	**Lat/Long**		**Stake height**
1	21.1298	149.18992	Left: 124cm, Right: 129.5cm
2	21.12983	149.18988	
3	21.1298	149.18989	
4	21.12989	149.1898	
5	21.12993	149.18976	
6	21.12994	149.18978	
7	21.13004	149.18966	
8	21.13007	149.1897	
9	21.3009	149.18963	
Last point	21.13014	149.18953	
BT3	**Barnes Creek**		
Plot #	**Lat/Long**		**Stake height**
1	21.13105	149.19222	Left: 76.5cm (DPI pole), Right:115cm
2	21.13099	149.19217	
3	21.13101	149.19224	
4	21.131	149.1922	
5	21.13096	149.19226	
6	21.13088	149.19228	
7	21.13084	149.19228	
8	21.1308	149.19231	
9	21.13077	149.19234	
10	21.13074	149.19238	
11	21.13069	149.19241	
12	21.13069	149.19254	
13	21.13064	149.19253	
BT4	**Barnes Creek (Continuation of BT3)**		
Plot #	**Lat/Long**		**Stake height**
1	21.13067	149.19258	
2	21.13055	149.19623	
3	21.13054	149.19256	
4	21.13047	149.19258	
5	21.13042	149.19264	
6	21.13035	149.19261	

BAKERS CREEK TRANSECTS

BCT1	Bakers Creek		
Plot #	**Lat/Long**		**Stake height**
1	21.21728	149.15997	Left: 121cm, Right: 127cm
2	21.21722	149.15988	
3	21.21719	149.15986	
4	21.21709	149.18986	
5	21.21705	149.15983	
6	21.21701	149.15981	
7	21.21698	149.15977	

BCT2	Bakers Creek		
Plot #	**Lat/Long**		**Stake height**
1	21.21173	149.19334	Left: 151.5cm, Right: 128 cm
2	21.21167	149.19328	
3	21.2117	149.19324	
4	21.21167	149.19322	
5	21.21167	149.19306	
6	21.21165	149.193	
7	21.21165	149.19297	
8	21.21161	149.19293	
9	21.21161	149.1929	
10	21.21553	149.19288	
11	21.21154	149.19283	

BCT3	Bakers Creek		
Plot #	**Lat/Long**		**Stake height**
1	21.21069	149.19333	
2	no signal		
3	"		
4	"		
5	"		
6	"		
7	"		
8	"		
9	"		
10	"		

APPENDIX 12: Locations and GPS references for water sampling sites the Pioneer River and McCreadys Creek.

WATER SAMPLING LOCATIONS

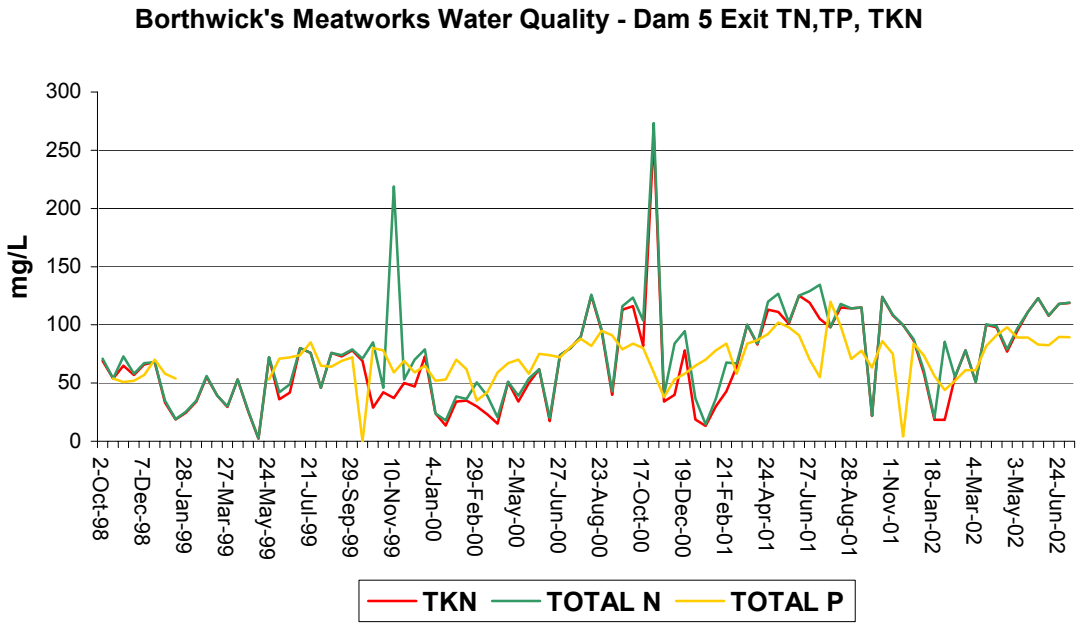
Site Code	Location	GPS references	
PW1	Pioneer River	21.12774	149.18052
PW2	Pioneer River	21.12778	149.18193
PW3	Pioneer River	21.13151	149.19805
PW4	Pioneer River	21.132	149.20689
PW5	Pioneer River	21.14008	149.21233
MCW1	McCreadys Creek	21.0761	149.17874
MCW2	McCreadys Creek	21.07519	149.18162
MCW3	McCreadys Creek	21.07297	149.1902
MCW4	McCreadys Creek	21.06935	149.19595
MCW5	McCreadys Creek	21.07232	149.19429

APPENDIX 13: Plot locations and stake heights in McCreadys Creek, Barnes Creek and Bakers Creek mangrove plots

PLOT LOCATIONS AND STAKE HEIGHT

Site Code	Location	Height (cm)	GPS references	
MCH1	McCreadys Creek	89	21.074775	149.18764
MCM1	McCreadys Creek	107	21.07455	149.18792
MCS1	McCreadys Creek	101	21.07492	149.18703
BH1	Barnes Creek	96	21.13031	149.19256
BM1	Barnes Creek	124	21.12975	149.18966
BS1	Barnes Creek	66	21.130085	149.18977
M1	Barnes Creek	85	0727124 E	7662340 N
M2	Barnes Creek	86	21.12891	149.19045
BCH1	Bakers Creek	126	21.21135	149.19326
BCM1	Bakers Creek	137	21.21154	149.19315
BCS1	Bakers Creek	127	21.21687	149.16024
BCS2	Bakers Creek	123	21.21147	149.19299

APPENDIX 14: Output of total nitrogen, total phosphorus and total kjeldahl nitrogen from the Borthwick's Meatworks in Bakers Creek



APPENDIX 15: Emissions from the Mount Bassett Sewerage Treatment Plant (Source: National Pollutant Inventory Database, 2002)

	Total Nitrogen in Water (kg)	Total Phosphorus in Water (kg)	Emission Reduction Activities
1 July 1998 to 30 June 1999	220000	66000	Methane burner connected to digesters
1 July 1999 to 30 June 2000	210000	69000	Studies being undertaken to upgrade or replace facility
1 July 2000 to 30 June 2001	160000	52000	None reported

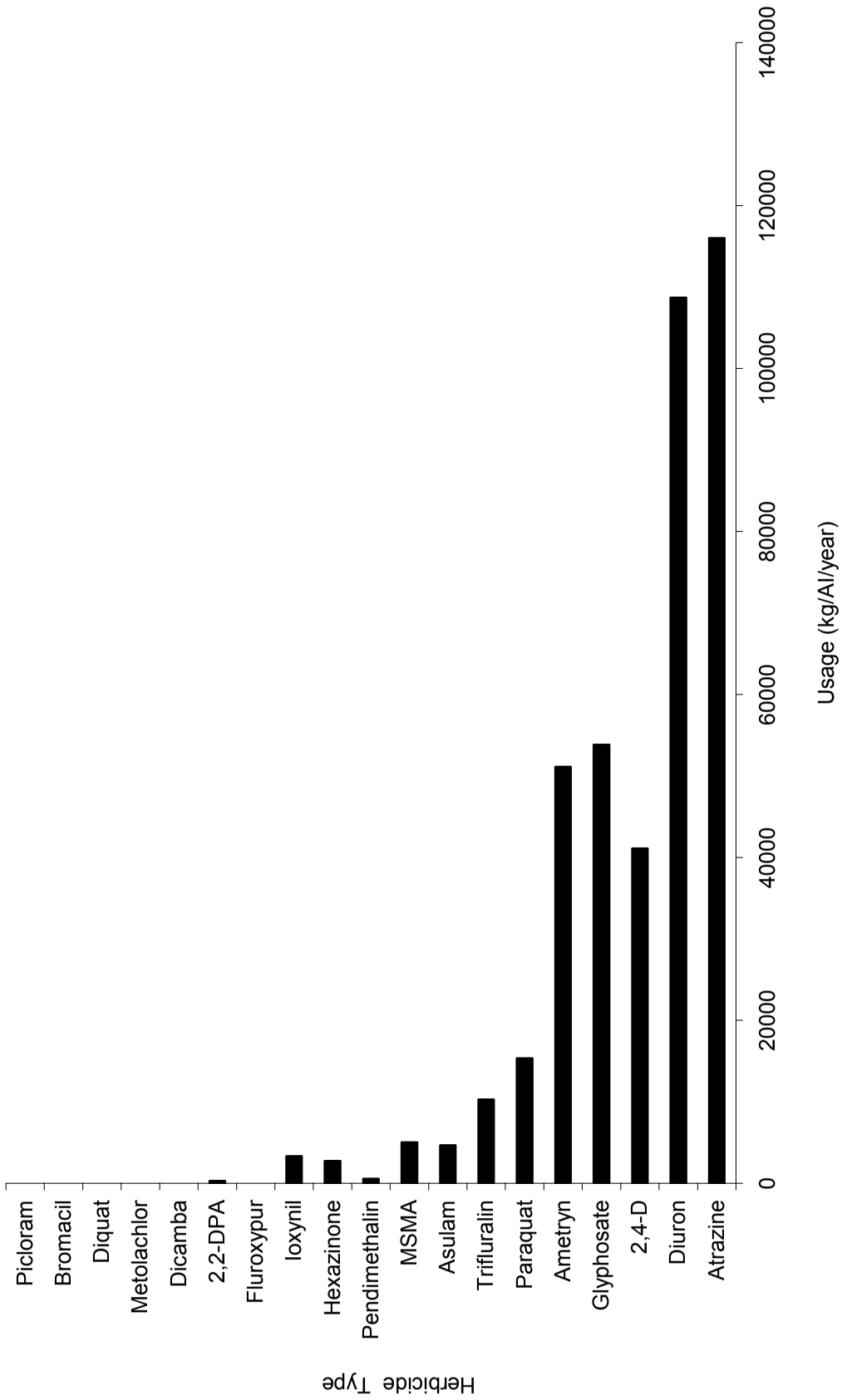
APPENDIX 16: Recommended sediment quality guidelines for metals and metalloids (mg/kg dw) (ANZECC, 2000).

	ISQG-Low (Trigger Value)	ISQG-High
Arsenic	20	70
Cadmium	1.5	10
Chromium	80	370
Copper	65	270
Lead	50	220
Mercury	0.15	1
Nickel	21	52

APPENDIX 17: Default trigger values for nutrients in tropical Australian estuaries for slightly disturbed ecosystems (ANZECC, 2000).

Total P (TP mg P/L)	Filterable Reactive P (FRP mg P/L)	Ammonia (NH_3 mg N/L)	Oxides of N (NOx mg N/L)	Total N (TN mg N/L)
0.02	0.005	0.006-0.0074	0.03	0.25

APPENDIX 18: Pesticide usage (kg/AI/year) in Queensland sugarcane areas in the Central Region (Source: Simpson *et al.*, 2001).



APPENDIX 19: Properties of the herbicides detected in mangrove surface sediments (0-5cm) in the Mackay region

A number of classes of PSII herbicides exist, including the ureas, triazines, anilides, triazinones, pyridazinones, benzimidazoles, *bis*-carbamates, phenylbiurets, cyanoacrylates, pyrones, cyclohexanediones, thiazolylidenketonitriles, naphthoquinones and nitrophenols (Bowyer *et al.,* 1991). The ureas and the triazines have been two of the most commercially important and popular classes of PSII herbicides since their introduction between 1950 and 1960 (Bowyer *et al.,* 1991). Three herbicides belonging to the triazine and ureas that are commonly used for pre- and pos-emergence control of weeds in agricultural applications in Queensland, Australia are atrazine, ametryn and diuron (Hamilton and Haydon, 1996, Simpson *et al.,* 2001a – See Appendix 13).

Atrazine (2-chloro-4-ethylamino-6-isopropylamino-1,3,5-triazine) is a triazine herbicide used for selective pre- and post- emergent control of annual grass and broadleaf weeds in a variety of vines, orchards, plantations and crops, particularly maize and sorghum (Crop Care, 2001; O'Grady and Sluggett, 2000). Atrazine is low to moderately soluble in water (33mg/L), with a moderate half-life, but does not absorb strongly onto soil particles, therefore there is potential to leach to groundwater and to move in runoff, in both dissolved and sediment-bound phases (Simpson *et al.,* 2001a). As a consequence, atrazine is frequently found in ground water beneath agricultural lands (Huber, 1993).

A freshwater moderate reliability trigger value of 13µg/L was derived for atrazine. It was considered preferable to adopt the freshwater figure as a marine low reliability trigger value. This should only be used as an indicative interim working level (ANZECC, 2000).

Ametryn (N^2-ethyl-N^4-isopropyl-6-methylthio-1,3,5-triazine-2,4-diamine) is a triazine herbicide used for pre- and post- emergent control of annual grasses and broadleaf weeds in pineapples, sugarcane, and non-crop situations (Crop Care, 1998).

Diuron (3-(3',4'-dichlorophenyl)-1,1-dimethylurea) is a selective urea herbicide used for pre-emergent and early post-emergent control of grass and broadleaf weeds (O'Grady and Sluggett, 2000). In Australia, diuron has around 2200 registered uses including application to pineapples, cotton, bananas, sugarcane, lucerne, cereals, citrus, vineyards, rights of way, commercial and industrial areas (Nufarm, 2001). Diuron is commonly found in the surface sediments (Hamiltion and Haydon, 1996).

A freshwater low reliability trigger value of 0.2µg/L was calculated for diuron using an AF of 200 on the lowest of a limited set of chronic data. A marine low reliability trigger value of 1.8µg/L was calculated for diuron using an AF of 1000. These figures should only be used as indicative interim working levels (ANZECC, 2000).

Hexazinone (3-cyclohexyl-6-dimethylamino-1-methyl-1,3,5-triazine-2,4(1*H*,3*H*)-dione) is a triazine herbicide used to control grasses and broadleaf and woody plants on tree plantations, sugarcane, pineapple and alfalfa (Tomlin, 1994). In Australia it is commonly used to treat woody weed species, as well as on a variety of weeds in pine plantations, commercial/industrial areas and rights-of-way (ANZECC, 2000). Hexazinone can be used as a mixture with diuron and is absorbed through the roots and/or leaves depending on type of formulation and method of application, however it is less mobile when taken up from the foliage. Hexazinone is persistent in water at pH 5,7 and 9, has a high solubility in water and is poorly absorbed to soil particles. It also has a moderate to high persistence in soil environment and therefore is a concern for groundwater contamination.

A freshwater low reliability trigger value of 75µg/L was calculated for hexazinone using an AF of 1000. In the absence of marine data, this was adopted as a marine low reliability trigger value. These figures should only be used as indicative interim working levels.

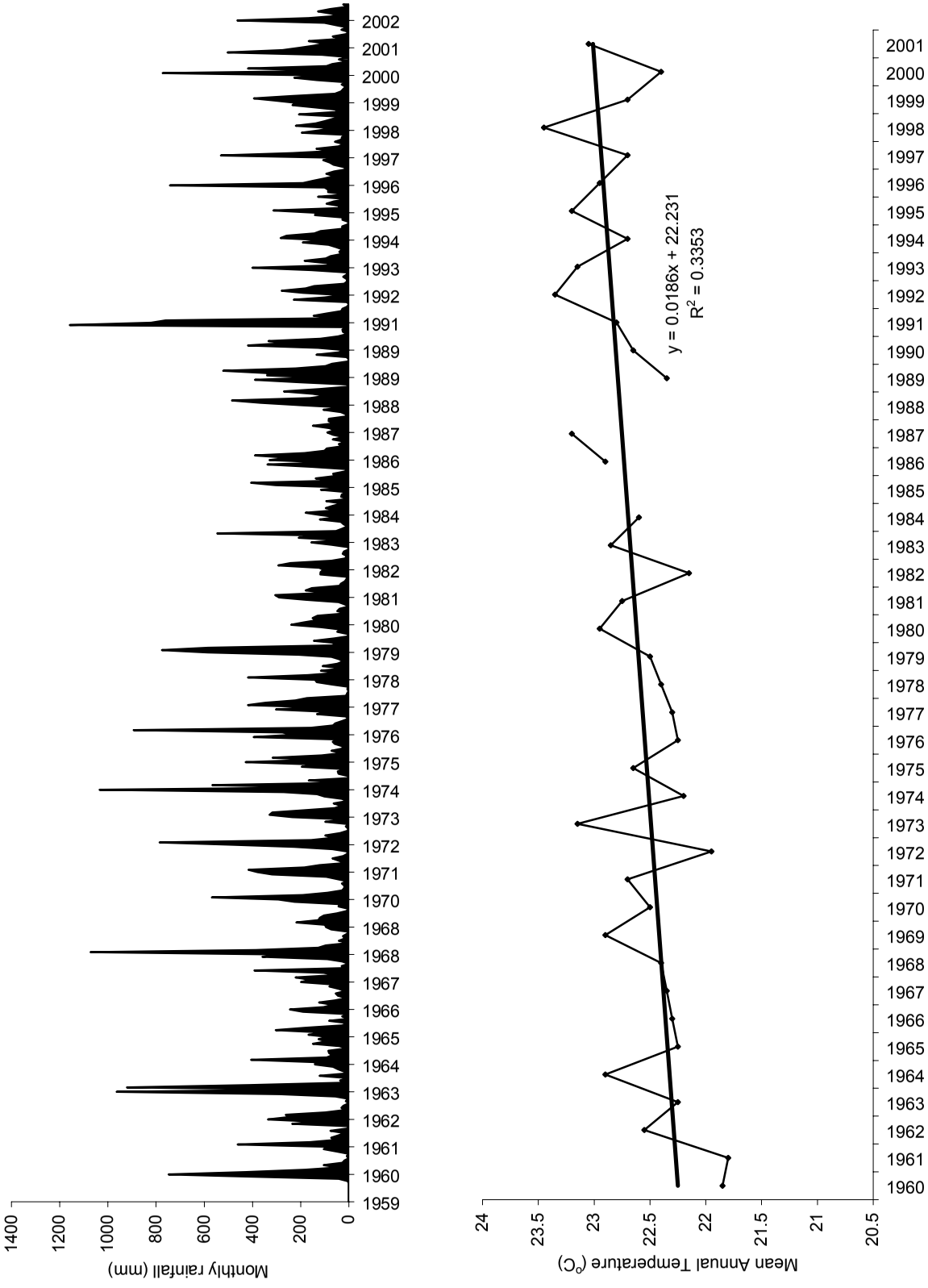
Tebuthiuron (1-(5-*tert*-butyl-1,3,4-thiadiazol-2-yl)-1,3-dimethylurea) is a broad spectrum herbicide used for control of woody and herbaceous plants in non-cropland areas (pastures, industrial areas), and rights-of-way. In Queensland, tebuthiuron can only be legally used in Eucalypt woodlands [broadleaf (silverleaf) ironbark. It is readily absorbed through roots and translocated to other plant parts. Tebuthiuron is highly persistent and has longer half-lives in drier areas or in soils with high organic matter content. Photodecomposition, volatilisation and evaporation are negligible.

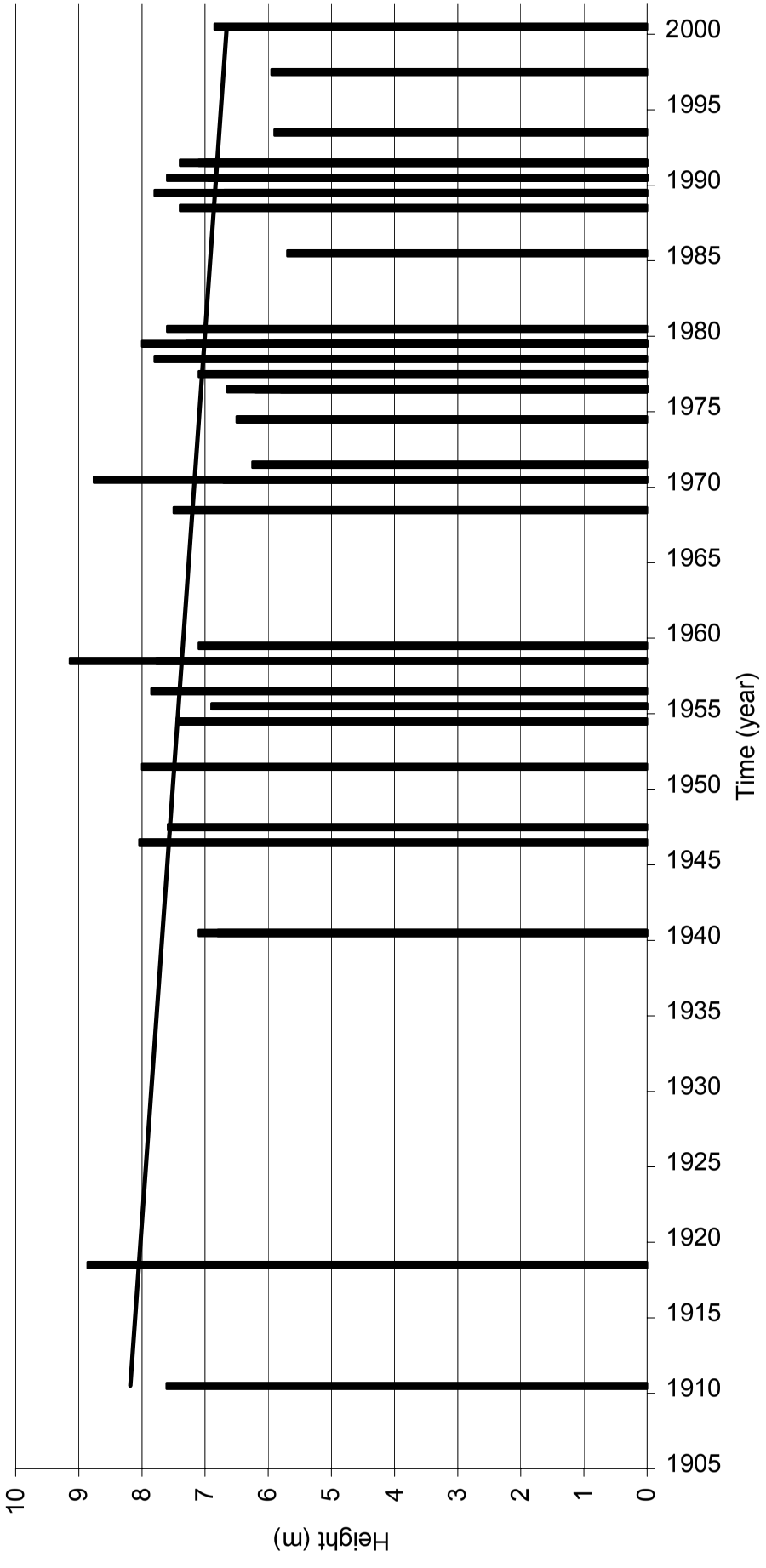
The freshwater high reliability trigger value of 2.2µg/L was derived for tebuthiuron. In the absence of marine data, the freshwater figure of 2.2µg/L was adopted as a marine low reliability trigger value. This figure should only be used as an indicative interim working level (ANZECC, 2000).

Simazine (6-chloro-N^2N^4-diethyl-1,3,5-triazine-2,4-diamine) is a triazine herbicide. Plants absorb simazine mainly through roots with little to no foliar penetration and accumulate it in the leaves and meristems. Simazine is used for control of a wide variety of grasses and broad-leafed weeds in fruit, vines, nuts, pineapples, vegetables, flowers, sugarcane, coffee, tea, turf and in forestry (Tomlin, 1994). It has over 2700 registered uses in Australia (NRA, 1997).

A freshwater moderate reliability trigger value of 3.2µg/L was derived for simazine. In the absence of marine data, 3.2µg/L was adopted as a marine low reliability trigger value. This should only be used as an indicative interim working level (ANZECC, 2000).

APPENDIX 20: Rainfall and temperature data for Mackay MO from the years 1959 to 2002 (Source: Bureau of Meteorology Australia, 2002)





APPENDIX 21: Pioneer River flood data from the years 1905 to 2000 (Source: Bureau of Meteorology Australia, 2002)