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# The use of the brown macroalgae, *Sargassum flavicans*, as a potential bioindicator of industrial nutrient enrichment

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ABSTRACT

Nutrient bioindicators are increasingly being recognised as a diagnostic tool for nutrient enrichment of estuarine and marine ecosystems. Few studies, however, have focused on field translocation of bioindicator organisms to detect nutrient discharge from industrial waste. The brown macroalgae, *Sargassum flavicans*, was investigated as a potential bioindicator of nutrient-enriched industrial effluent originating from a nickel refinery in tropical north-eastern Australia. *S. flavicans* was translocated to a number of nutrient enriched creek and oceanic sites over two seasons and assessed for changes in stable isotope ratios of $^{15}N$ and $^{13}C$ within the plant tissue in comparison to reference sites. Nutrient uptake in macroalgae, translocated to the nutrient enriched sites adjacent to the refinery, increased 3–4-fold in $\delta^{15}N$, compared to reference sites. Using $\delta^{15}N$ of translocated *S. flavicans* proved to be a successful method for monitoring time-integrated uptake of nitrogen, given the current lack of passive sampler technology for nutrient monitoring.



## 1. Introduction

Estuarine and marine concentrations of Dissolved Inorganic Nitrogen (DIN), particularly in the form of ammonia, nitrate and nitrite, have steadily increased due to anthropogenic activity (Ahad et al., 2006; Eddy, 2005; Harris, 1999; Liu et al., 2007). DIN can originate from a number of sources, including urban and agricultural activities, aquaculture facilities, and Waste Water Treatment Plants (WWTP) (Costanzo et al., 2003; Lapointe et al., 2010; Lin and Fong, 2008; Stephens and Farris, 2004; Volkman et al., 2007), affecting natural nitrogen cycling, phytoplankton communities and lower trophic level stability (Balata et al., 2008; Bishop et al., 2006; Burkholder et al., 2007; Camargo and Alonso, 2006; Dolbeth et al., 2007; Fabricius, 2005). Moreover, increased DIN inputs into adjacent estuarine systems from industrial inputs, such as pulp mills, mining and refining processes, are consistent point sources of DIN, which require regulatory monitoring and assessment for the maintenance of the neighbouring estuarine and marine environments (Bothwell, 1992; Camargo and Alonso, 2006; Church et al., 2006; Friese et al., 1998; Scrimgeour and Chambers, 2000; Seitzinger et al., 2002; Woelfl et al., 2000).

Given the dynamic and varying nature of the hydrology and underlying water characteristics in tropical estuarine and marine environments, which are mainly influenced by local geology, eco-hydrology, micro-climates, and more broadly, seasonal changes, traditional, *in situ,* water quality sampling only represents a snapshot in time. Chemcatchers® and passive samplers can quantify certain chemicals, such as metals and pesticides within the water column over time (Don and Vroblesky, 2007; Greenwood et al., 2007; O'Brien et al., 2012; Vermeirssen et al., 2009; Warnken et al., 2007), however, there is a deficiency in passive sampler technologies that can be used to determine nutrient levels in the water column over specific time periods. The use of indicator species benefits over traditional grab samples in that the extended period of deployment allow nutrient concentrations within the water column to be accumulated in the indicator organisms, providing a calculable time-averaged measurement of bioavailable nutrients in waters over a period of several days.

A number of natural floral and faunal bioindicators such as macroalgae, mangroves, mussels, earth worms, seagrass, and corals, have been used in the past to detect nutrient enrichment from sewage treatment facilities, shrimp farms, and agricultural runoff (Costanzo et al., 2003,2004; Lin and Fong, 2008; Risk et al., 2009; Schmidt and Ostle, 1999). On the other hand, very few studies have used manipulative translocation techniques to detect anthropogenic inputs, such as industrial nutrient inputs, into an estuarine or marine environment (Costanzo et al., 2005; Fertig et al., 2009;

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Udy and Dennison, 1997). The advantages of using translocation techniques include the fact that the translocated species has not been previously exposed to local or regional contaminants; all sites contain algae from the same remote source site, reducing spatial variability; and a 'before and after' exposure estimate can therefore be accurately obtained.

For the study described below, the use of the brown macroalgae, *Sargassum flavicans*, was examined as a potential bioindicator of industrial nutrient enrichment using stable isotope analysis, by translocating the algae into areas of known higher concentrations of DIN. A number of algae species have successfully been used as bioindicators of estuarine and marine nutrient enrichment (eutrophication) (Pihl et al., 1999; Smith, 1996), by using stable isotope ($\delta^{15}N/\delta^{13}C$) signatures (Costanzo et al., 2005; Rogers, 2003). Stable isotope signatures have also successfully been applied as tracers of nutrient enrichment on flora and fauna, particularly with respect to algae and algal grazers such as limpets and mussels (Fry and Allen, 2003; Gray, 2002; Rogers, 2003). Higher order plants, such as mangroves, have been used as longer term biomonitors of nutrient enrichment (Costanzo et al., 2004). Changes in carbon and nitrogen ratios in organisms can identify prominent nutrient sources (Peterson and Fry, 1987; Risk et al., 2009). *S. flavicans* was selected due to its favourable attributes as a bioindicator species, given that the species is readily available in subtropical and tropical estuarine and marine systems, is available all year round, and shares similar traits with other *Sargassum* species such as being a nutrient opportunist and sensitive to nutrient uptake (Matsuo et al., 2009; Roberts et al., 2008; Rossi et al., 2009; Yuka et al., 2001). The geographic range of *S. flavicans* is extensive, as it can be found in Southwest Asia, Africa, Southeast Asia, Australia and New Zealand and the Pacific Islands (Phillips, 1995).

This study was conducted in Halifax Bay, situated to the north of the city of Townsville (19° 15.48′S, 146° 49.09′E) and south of the town of Ingham (18° 39.07′S, 146° 9.31′E) in far north Queensland, Australia (Fig. 1). The bay forms part of the Great Barrier Reef World Heritage Area and the Wet Tropics World Heritage Area. There are a number of ephemeral tropical creeks and rivers that flow into Halifax Bay, which receive inputs from a variety of coastal catchments from both mixed land use and industry. The majority of the land adjacent to Halifax Bay consists of dry sclerophyll forest and patches of rainforest in the upper catchments, with very little urban development, and only a number of small beach communities. Apart from a refinery, there are negligible to very low amounts of other industrial nutrient discharges within the bay (Longstaff et al., 2001). The Burdekin River, which is located approximately 120 km south of Halifax Bay, can intermittently discharge large amounts of sediments, nutrients and toxicants during significant wet seasons, causing elevated nutrient rich and turbid water on a regional scale (Bainbridge et al., 2012). Industry refining processes use a number of technologies to extract metals from ore. For example, nickel and cobalt can be selectively extracted by using concentrated ammonia, in the form of concentrated ammonium carbonate liquor, to separate the metals as ammine complexes from the ore (Forbes et al., 2000; Jana and Akerkar, 1989; Pandey and Kumar, 1991; Price and Reid, 1993). Given the large quantities and high concentrations of ammonia as part of the refining process, particularly for cobalt and nickel extraction, ammonia may become an artificial footprint of nickel refineries if sufficient quantities of ammonia inadvertently find its way into the adjacent receiving environment of such facilities. The current study was implemented in 2010, close to a nickel/cobalt refinery, to determine novel techniques on the use of indicator species to monitor nutrient inputs using a time integrated approach.

The objectives of this study were to determine the usefulness of the brown algae, *S. flavicans*, as a potential bioindicator of estuarine and marine nutrient loads by use of stable isotope analysis. The specific aims of the study were to (a) determine changes in nutrient uptake in *S. flavicans* along known nutrient enriched gradients, compared to reference sites, (b) compare differences among creek sites and oceanic sites, and (c) determine differences between wet and dry season uptake in a tropical marine coastal environment.

## 2. Methods

### 2.1. Sampling design

In order to determine the usefulness of *S. flavicans* as a potential bioindicator of nutrient enrichment, a series of creek and oceanic sites were established in the receiving environment adjacent to industrial infrastructure and in reference locations. The study was conducted in Halifax Bay, northern Queensland, Australia (19°03′ S, 146°29′ E). A total of ten sites were established, five creek sites and five oceanic sites (Fig. 1). Experiments were undertaken during the 2010 wet season (February/March) and revisited during the dry season of June 2010 to determine seasonal changes. Creek sites included three sites adjacent to an industrial refinery (AC, BC, HC), and two reference sites, (SW and RC). In order to compare creek nutrient concentrations with nutrient concentrations within Halifax Bay, five oceanic sites were established. Two sites adjacent to the refinery (C2 and B2) as well as three reference sites (K2, G2 and I2) (Fig. 1).

### 2.2. Sample collection

The brown marine macroalgae, *S. flavicans*, was collected from a relatively low nutrient, oligotrophic location (<3‰ $\delta^{15}N$) (Moss et al., 2005; Waycott et al., 2005; Webster et al., 2005), located on an outer coastal fringing reef located offshore of Gladstone, Queensland, Australia (23°57′ S, 151°29′ E). *S. flavicans* was collected by SCUBA and stored in seawater in a large aerated insulated polyethylene container during travel. The highest (youngest) part of the algae was used for the experiment in order to obtain maximum uptake rates, given that nutrient uptake in *Sargassum* algae increases with height due to raised photosynthetic activity (Ishihi et al., 2001).

The algae was transported to Townsville from Gladstone and deployed within 12 h of collection. A sub-sample of algae was collected from the site of origin (off Gladstone) and frozen for further analysis to determine background levels of stable isotopes (denoted; Reference or REF). A second sub-sample was collected and frozen after algae were deployed at all sites in Townsville to determine if any changes in stable isotopes occurred during transport (denoted; Transport Control or TC).

Algae samples (about 50 g fresh weight) were translocated and deployed (transplanted) at sites close to the mouth of the creeks and at predetermined oceanic sites, approximately 2 km offshore, in triplicate, using small clean acid-washed polypropylene containers at each site for an, *in situ,* deployment period of 120 h (5 days) suspended one meter below the water surface using an Aqua Buoy 600 (Costanzo et al., 2005). After the fifth day (120 h), samples were collected and frozen for laboratory based analyses. A further reference site was established at the location where the algae was initially collected, off Gladstone, and re-deployed at the same collection location using the above mentioned method for 120 h. This exercise was conducted to determine if cutting and re-deploying the algae using the polypropylene containers would have an effect on isotope levels (denoted; Cutting control or CC).

In order to understand the relationship between nutrient enrichment in macroalage and water column nutrient loads, nutrient water samples were collected at the same times from creek sites during both seasons. Triplicate water samples were collected