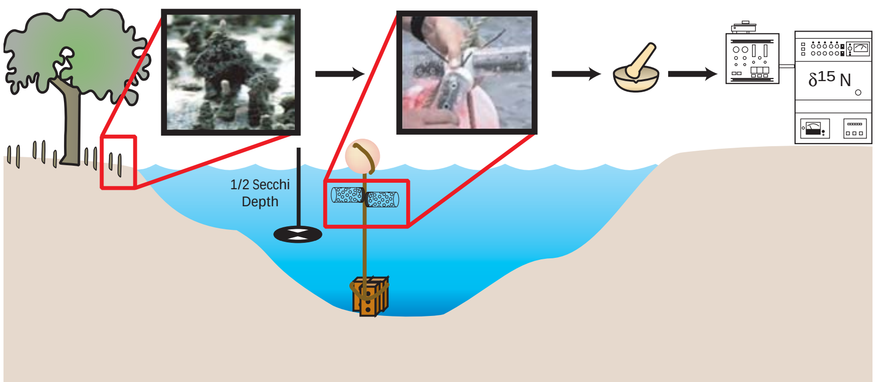
ASSESSING NUTRIENT SOURCES

This Integration and Application Network newsletter explores the assessment of nutrient sources using stable isotope signatures of various marine organisms. This technique was developed in Moreton Bay, Australia for mapping sewage plumes, and was also used to determine the extent of aquaculture effluent (shrimp ponds) and to distinguish agricultural runoff (sugar cane) from other sources. Most of the analyses were conducted with macroalgae, but oysters, seagrass and mangroves were also utilized. Sewage plume mapping has now been incorporated into the ongoing monitoring program in Moreton Bay (see www.healthywaterways.org). The application of this technique for Chesapeake Bay is being tested in the Choptank and Patuxent Rivers using a variety of organisms (e.g. clams, macroalgae, and seagrasses).



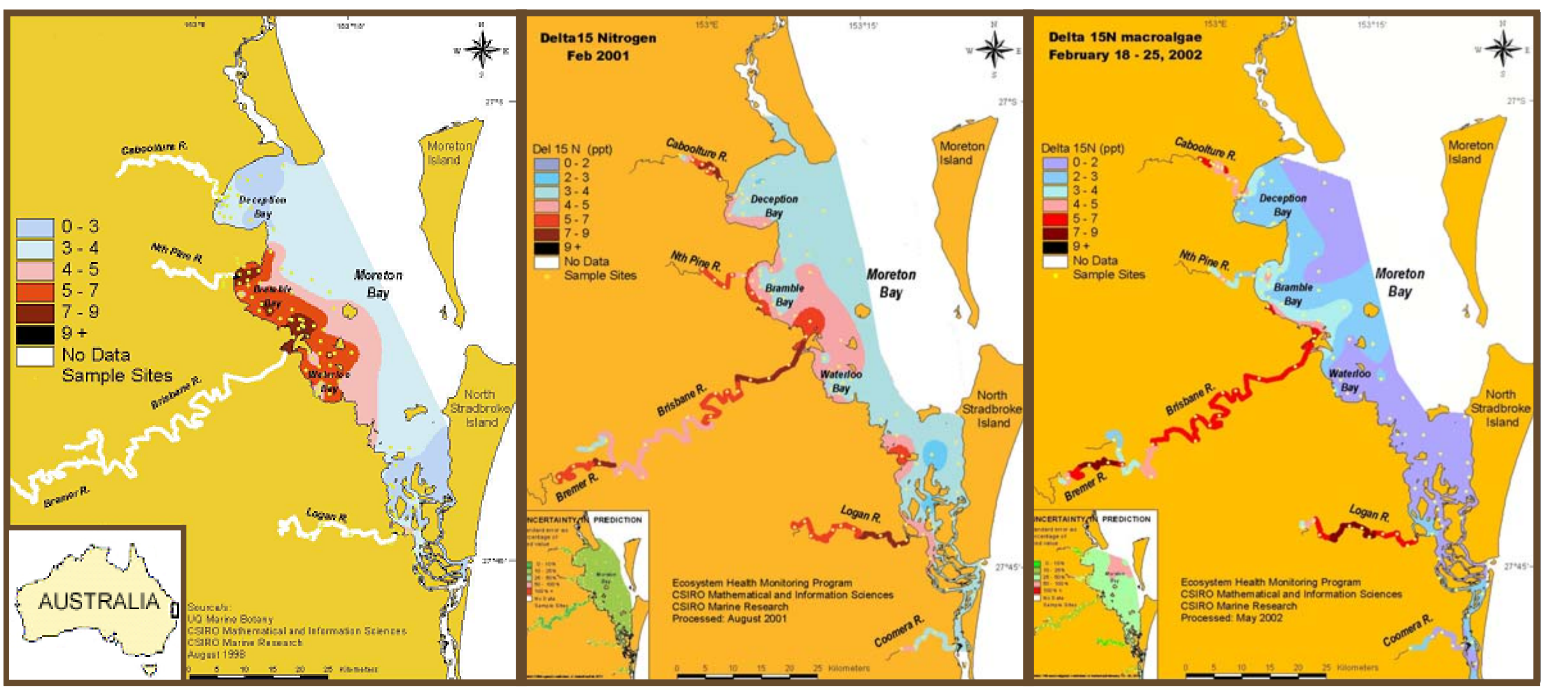
Sewage plume mapping process: *Collect macroalgae (or other indicator organisms such as oysters, bivalves or submerged aquatic vegetation) from a site distant from nutrient sources, incubate in situ, then dry, grind and analyze on a stable isotope mass spectrometer for determination of* $\delta^{15}N$.

WHAT IS $\delta^{15}N$?

There are two naturally occurring atomic forms of nitrogen (N), ^{14}N *and* ^{15}N*, with 0.3663% of atmospheric N in the heavy form. Plants and animals assimilate both forms of nitrogen, and the ratio of* ^{15}N *to* ^{14}N *compared to an atmospheric standard (*$\delta^{15}N$*) can be determined by analysis of tissue on a stable isotope mass spectrometer using the following equation:*

$$\delta^{15}N = [(\text{atom \% sample} - \text{atom \% standard})/\text{atom \% standard}]*1000$$

In regions subject to anthropogenic inputs of nitrogen changes in the $\delta^{15}N$ *signature can be used to identify the source and distribution of the nitrogen.*



Images: *During the summer of 1998 (left), sewage plumes emanated from the mouths of two rivers with large sewage treatment plant discharges. In the summer of 2001 (middle), two smaller, distinct plumes can be seen, and the addition of sites in the rivers shows the sewage nitrogen mostly restricted to the rivers. By summer of 2002 (right), the sewage plumes have been greatly reduced, mostly restricted to the Brisbane river.*

Data courtesy of Ben Longstaff, Ecosystem Health Monitoring Program. Website: http://www.coastal.crc.org.au/ehmp/

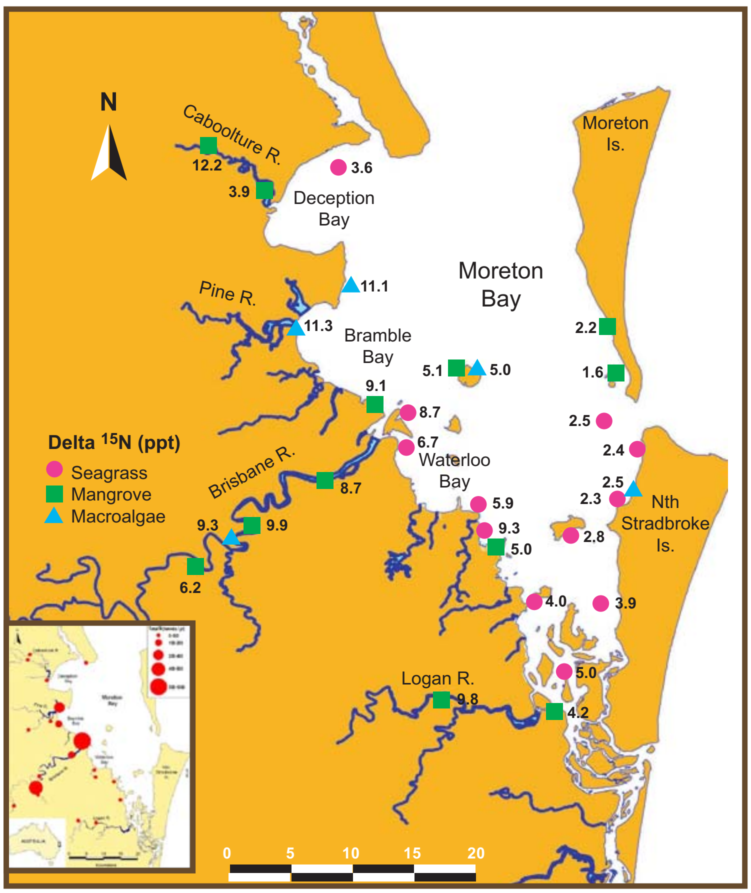
SEAGRASS, MACROALGAE & MANGROVES

$\delta^{15}N$ can be measured in different species

Nitrogen (N) discharge from sewage treatment plants was identified from analysis of ambient algae, seagrass, mangrove and macroalgae samples. The elevated signature detected in the sediment identified that sewage N was available in the environment. The presence of the elevated signatures in the plant bioindicators distinguished that the N was incorporated into the vegetation.

$\delta^{15}N$ signatures of marine plants were highest when grown in the vicinity of sewage outfalls within the rivers and the estuarine portions of the Moreton Bay. At sites adjacent to sewage treatment plants (STPs) in the rivers, the $\delta^{15}N$ signatures of mangrove leaves were greater than 9. In the bay, at sites adjacent to STPs, mangrove leaf values were 9.1 at the Brisbane River mouth and 11.3 (for macroalgae) at the Pine River mouth, while in the eastern bay, the mangrove leaf $\delta^{15}N$ signature was as low as 1.6. These values demonstrate the strong influence of sewage in the rivers and western bay near to sewage discharges.

This map, resulting from a variety of bioindicators, provides support for interpretation of nutrient sources, but demonstrates the relative differences between the species used. Standardizing the bioindicator species used is an important component of an assessment program.



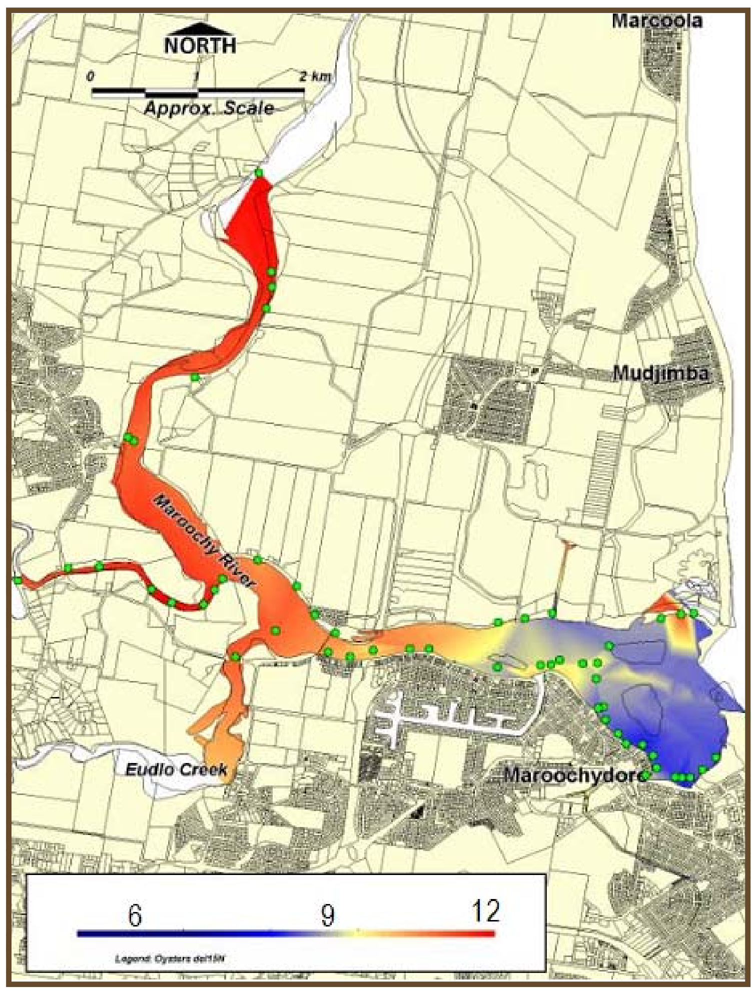
__Passive bioindicator:__ Ambient $\delta^{15}N$ signatures of marine plants in Moreton Bay. High $\delta^{115}N$ signatures were found near sewage discharges, while at sites distant to sources, signatures were low.

__Inset:__ Location and size of sewage treatment plants in Moreton Bay. The relative size of the red dots represents the total N

ACTIVE VS PASSIVE BIOINDICATORS

The collection of macrobiota (including algae, mangroves and seagrasses) allows detection of the $\delta^{15}N$ signature from plants which over time have integrated the signature of their environment. These are passive indicators as they incorporate the signature in their natural environment throughout their growth cycle. However, there are many sites in which these biological indicators are not available for collection such as in the open water and in degraded areas. Here, biological plant indicators may be actively deployed to incorporate the signature over smaller time periods (days). These are therefore active indicators which provide a view of the $\delta^{15}N$ of the environment at the time in which the indicator was deployed. The results of active sampling varies at different sampling times and therefore provides insight into temporal variation (such as seasonal) of the extent of sewage nitrogen.

OYSTERS: Most $\delta^{15}N$ measurements are of plants that directly absorb nutrients. However, the uptake of nutrients by phytoplankton which are then filtered by oysters, provides a $\delta^{15}N$ signature within oysters that reflects nutrient sources in the ecosystem.



__Passive bioindicator:__ Observed $\delta^{15}N$ of oysters reflects sewage and septic inputs in the Maroochy River, Australia.

POINT & NON-POINT SOURCES

$\delta^{15}N$ signatures may identify different nutrient sources

DETECTING DIFFERENT NUTRIENT SOURCES

The various sources of nitrogen pollution to coastal ecosystems often have distinguishable $^{15}N/^{14}N$ ratios (Heaton 1986).

Nitrogen fertilizer, produced by industrial fixation of atmospheric nitrogen results in low to negative $\delta^{15}N$ signatures.

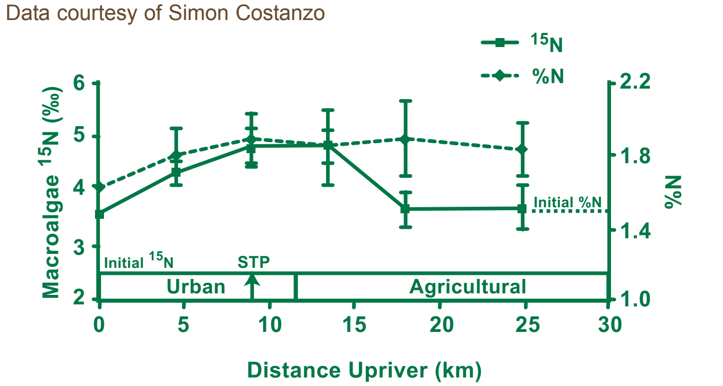
In animal or sewage waste, nitrogen is excreted mainly in the form of urea, which favours conversion to ammonia and enables volatilization to the atmosphere. Resultant fractionation during this process leaves the remaining ammonium enriched in ^{15}N.

Further biological fractionation results in sewage nitrogen having a $\delta^{15}N$ signature of ~10‰. Septic undergoes less biological treatment and is likely to have a signature closer to that of raw waste (~6‰).

The approach of using biological indicators (bioindicators) has several advantages:

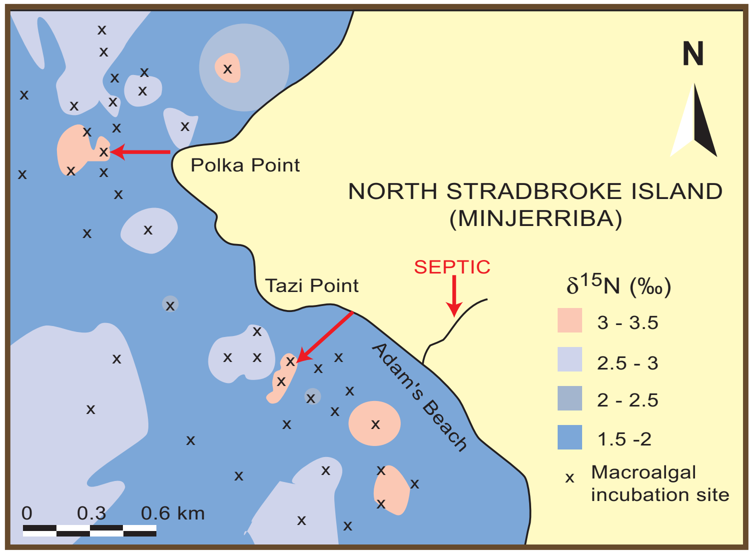
- **Integration over time:** marine organisms assimilate nutrients for use in metabolism and growth which are manifested as measurable changes over the life span of the organism, from days to years.
- **Bioavailable nutrients:** only those forms of nutrients that are available for uptake and assimilation by organisms are measured.
- **Sensitive:** bioindicators can detect very low nutrient concentrations and non-steady state conditions (eg. pulsed) that would go undetected by traditional sampling.
- **Interpretive power:** nutrient bioindicators can be used to infer source of nutrients and ecosystem impacts of nutrient enrichment.

Data courtesy of Simon Costanzo



Tweed River transect in which $\delta^{15}N$ signatures of agricultural inputs (sugar cane) can be distinguished from urban inputs.

Data courtesy of Simon Costanzo



$\delta^{15}N$ is sensitive at small spatial scales and effective at detecting small sewage sources (see red arrows) as well as septic discharges.

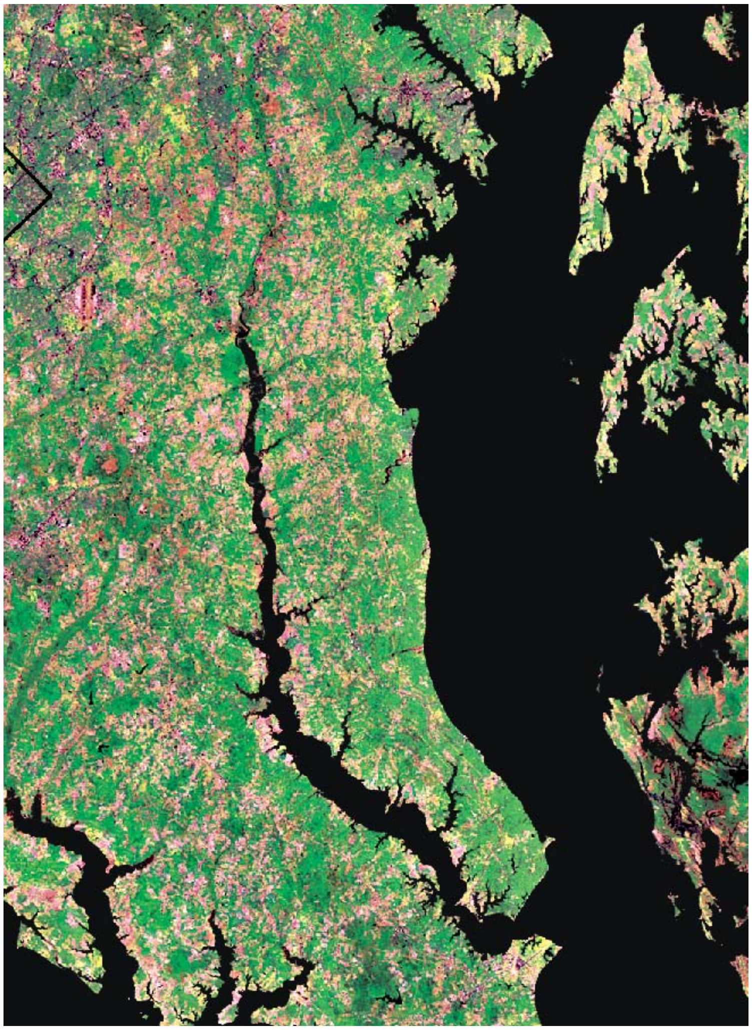
Agricultural inputs ($\delta^{15}N$ ~ 0-1)

Aquaculture discharge ($\delta^{15}N$ ~ 5+)

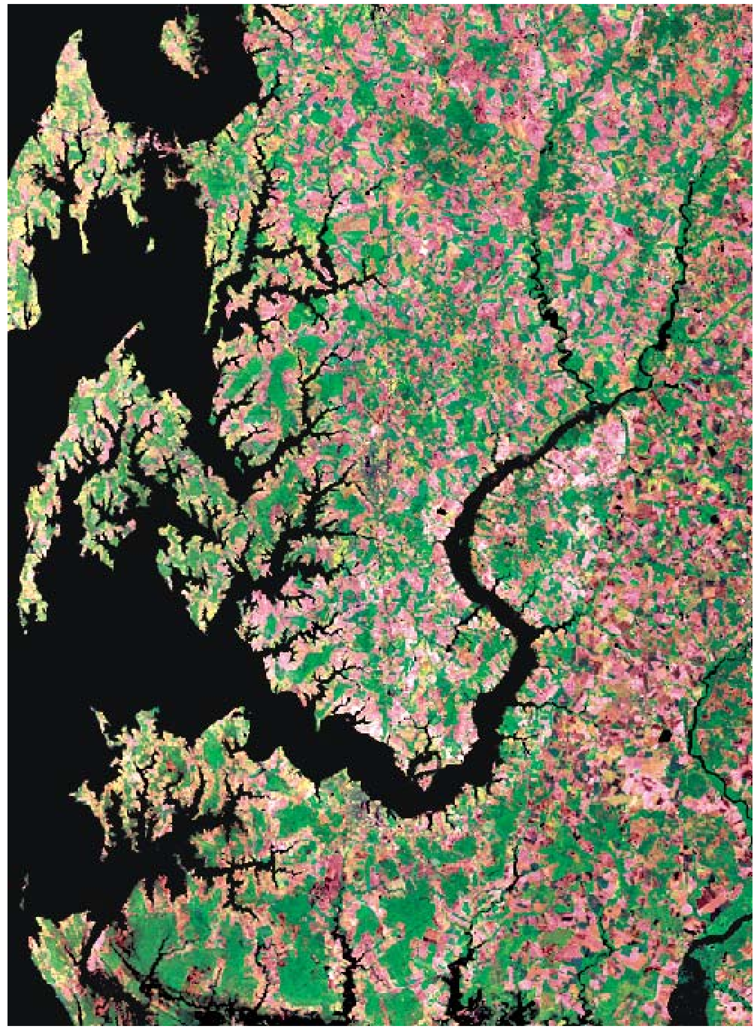
Sewage treatment plant ($\delta^{15}N$ ~ 9)

CHESAPEAKE BAY SEWAGE PLUME MAPPING

Patuxent and Choptank Rivers



Patuxent River (sewage and septic dominated)



Choptank River (agricultural dominated)

Images courtesy of USGS http://chesapeake.usgs.gov/images/cbimag2.jpg

The Choptank River on the Eastern Shore of Chesapeake Bay is largely surrounded by agricultural land, with several sewage treatment plants discharging into the river. In contrast, the Patuxent River on the Western Shore of Chesapeake Bay is largely surrounded by forested lands with suburban development, with most of the sewage discharged upstream. Nutrient sources for these two river systems also include atmospheric inputs. The stable isotope analysis approach will attempt to distinguish the various sources throughout each of the river systems.

*The **I**ntegration and **A**pplication **N**etwork (IAN) is a collection of scientists interested in **solving**, not just studying environmental problems. The intent of IAN is to inspire, manage and produce timely syntheses and assessments on key environmental issues, with a special emphasis on Chesapeake Bay and its watershed. IAN is an initiative of the faculty of the University of Maryland Center for Environmental Science, but will link with other academic institutions, various resource management agencies and non-governmental organizations.*

PRIMARY OBJECTIVES FOR IAN

- **Foster** problem-solving using integration of scientific data and information
- **Support** the application of scientific understanding to forecast consequences of environmental policy options
- **Provide** a rich training ground in complex problem solving and science application
- **Facilitate** a productive interaction between scientists and the broader community

Further Reading:

Abal EG, Dennison WC & Greenfield PF (2001) Managing the Brisbane River and Moreton Bay: An integrated research/management program to reduce impacts on an Australian estuary. *Water Science and Technology* 43: 57-70.

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Costanzo SD (2001) *Development of indicators for assessing and monitoring nutrient influences in coastal waters.* PhD dissertation, University of Queensland, Australia. 151pp.

Costanzo SD, O'Donohue MJ, Dennison WC, Loneragan NR & Thomas MT (2001) A new approach for detecting and mapping sewage impacts. *Marine Pollution Bulletin* 42: 149-56.

Dennison WC, Abal EG (1999) *Moreton Bay Study: A scientific basis for the Healthy Waterways campaign.* Brisbane: South East Queensland Regional Water Quality Management Strategy. 245 pp.

Heaton THE (1986) Isotopic studies of nitrogen pollution in the hydrosphere and atmosphere: a review. *Chemical Geology* 59: 87-102.

Jones AB, Dennison WC, Stewart GR (1996) Macroalgal responses to nitrogen source and availability: Amino acid metabolic profiling as a bioindicator using *Gracilaria edulis* (Rhodophyta). *Journal of Phycology* 32: 757-66.

Jones AB, O'Donohue MJ, Udy J, Dennison WC (2001) Assessing ecological impacts of shrimp and sewage effluent: biological indicators with standard water quality analyses. *Estuarine, Coastal and Shelf Science* 52: 91-109.

Udy JW, Dennison WC (1997) Physiological responses of seagrasses used to identify anthropogenic nutrient inputs. *Marine and Freshwater Research* 48: 605-14.

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INTEGRATION APPLICATION NETWORK