

University of Melbourne

# Seagrass Resilience in Port Phillip Bay

Final Report to the Seagrass and Reefs Program for Port Phillip Bay

Gregory Jenkins and Michael Keough  
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# **Seagrass and Reefs**

## **Final Report**

### **Seagrass Resilience in Port Phillip Bay**

Gregory Jenkins<sup>1</sup>, Michael Keough<sup>1</sup>, David Ball, Perran Cook<sup>2</sup>, Angus Ferguson<sup>3</sup>,  
Jennita Gay<sup>1</sup>, Alastair Hirst<sup>4</sup>, Randall Lee<sup>5</sup>, Andrew Longmore<sup>1</sup>, Peter Macreadie<sup>4</sup>,  
Sasi Nayar<sup>7</sup>, Craig Sherman<sup>4</sup>, Tim Smith<sup>1</sup>, Jeff Ross<sup>8</sup>, Paul York<sup>9</sup>

<sup>1</sup>School of BioSciences, University of Melbourne VIC 3010

<sup>2</sup>Water Studies Centre, School of Chemistry, Monash University, Clayton, VIC 3800

<sup>3</sup>NSW Office of Environment and Heritage, Sydney NSW 2000

<sup>4</sup>School of Life and Environmental Sciences, Deakin University, Geelong, VIC 3220

<sup>5</sup>Marine Water Environmental Solutions, Environmental Protection Authority, Macleod VIC 3085

<sup>6</sup>School of Life and Environmental Sciences, Deakin University, Burwood, VIC 3125

<sup>7</sup>SARDI- Aquatic Sciences, Henley Beach, SA 5022

<sup>8</sup>Institute for Marine and Antarctic Studies, University of Tasmania, Hobart, TAS 7005

<sup>9</sup>Centre for Tropical Water and Aquatic Ecosystem Research, James Cook University, Townsville, QLD 4811

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## Summary

Seagrass in Port Phillip Bay is dominated by the eelgrass, *Zostera nigricaulis*, which occurs around the margin of the bay from the shallow subtidal zone to depths of up to 8 metres. *Zostera* provides crucial ecosystem services such as stabilising sediments and improving water quality, reducing coastal erosion, and increasing biological productivity for the marine food chain as well as providing nursery habitats for key recreational and commercial fish species.

At the scale of the whole bay our research shows that the broad distribution of *Zostera* can be explained by two main factors, wave exposure (*Zostera* does not occur in areas of high wave energy) and depth (a proxy for the light reaching seagrass for growth). Within the broad area where *Zostera* can grow, however, there are regional areas of presence or absence and variation over time that cannot be explained by this simple model, and are likely influenced by limiting factors such as nutrients, turbidity affecting light, and sediment movement.

*Zostera* in Port Phillip Bay can obtain nutrients from a number of sources. Nutrients can be obtained directly in dissolved form from the water column or sediments (pore-water). These nutrients can come from sources such as river catchment, sewage treatment, Bass Strait and the atmosphere. Our experiments showed that nutrients can be taken up above ground (leaves) and below ground (roots and rhizomes) and can move between these compartments. Nutrients can also be taken up from the sediments indirectly through the bacterial breakdown of detritus that releases nutrients for uptake by seagrass. This detritus does not only come from dead seagrass but also algae, including phytoplankton (single-cell plants floating in the water column) that are “trapped” by the seagrass bed (reduced waves and currents in seagrass beds lead to phytoplankton settling into the bed). Finally, it is possible for seagrass to utilise Nitrogen in the gaseous form when bacteria associated with the root/rhizome system ‘fix’ atmospheric Nitrogen (although this pathway only meets a minor part of the plants’ nutrient needs).

Our studies on the influence of nutrients and sediments using modelling, chemical (stable isotope) analyses and experiments have shown that there are three broad categories of *Zostera* habitat within the bay. Areas of *Zostera* that are protected from current and wave exposure, and relatively isolated from the catchment, bay such as Swan Bay and Corio Bay, have relatively stable cover of seagrass over time (‘persistent’ seagrass beds). These seagrasses live in fine, muddy sediments, and most of their nutrients come from internal breakdown and recycling of detritus. In contrast, seagrasses living in more exposed parts of the bay, particularly the Bellarine Bank and the southern areas of the bay, have shown major increases and declines since the middle of last century (‘ephemeral’ seagrass beds). Our field and experimental studies indicate that these seagrass beds are nutrient limited, and major losses of *Zostera* occurred in these areas during the Millennium drought when catchment inputs of nutrients were low. Our studies also show that these areas have dynamic sediment movements and these may have also changed in response to climatic shifts during the drought, affecting seagrass distribution. The third category of seagrass habitat occurs along the north-west coast of the bay where nutrients are derived from the Western Treatment Plant and are unlikely to be limiting, but the combination of fine sediments and wave exposure means that turbidity is often relatively high and limiting for seagrass growth.

These different categories of *Zostera* habitat have differing levels of resilience to changes in water quality parameters such as nutrients and sediments. ‘Persistent’ beds are largely independent of changes to catchment and other inputs, and sediment transport processes, and as such are relatively resilient. In contrast ‘ephemeral’ beds are quite sensitive to changes in catchment inputs and sediment transport processes and will be expected to continue to show major variability over time in relation to climate and other factors.

The demography of *Zostera* also varies around the Bay, and we found evidence of separation of seagrass populations around the Bay. Demographic variation (and population segregation) are a mixture of regional differences and more localised, site specific ones.

At a broad scale, seagrasses responded to small-scale disturbances, including loss of leaves, loss of leaves and below-ground parts, in a broadly consistent way. Leaf regrowth was rapid, as was the extension of rhizomes from neighbouring areas into the disturbed area. When regrowth was prevented, recovery slowed dramatically, as we saw few signs of successful colonisation by seeds or drifting fragments. While we saw this general pattern everywhere, there were big differences between individual sites in the speed of this recovery, suggesting that some areas are less resilient than others. These differences did not match the broad regional patterns that we saw elsewhere. As a small side project, we also investigated, for two quite different seagrass sites, whether small disturbances caused the loss of “Blue Carbon”, but we saw little change.

Seagrass reproduction occurred from mid-spring through to early summer, with peak flowering in October. There were small differences around the bay in the timing of flowering, but big differences in the amount of flowering, numbers of seeds produced, and numbers of seeds buried in the sediment. We saw consistent, extensive flowering in the Geelong Arm, consistent, but less extensive flowering in Swan Bay, and limited flowering at the north end of the Bay. In the southeast, a seagrass meadow at Blairgowrie flowered extensively, and we saw some build-up of the seed bank.

We used genetic tools to examine patterns of sexual and asexual reproduction within seagrass meadows and also to infer dispersal between different areas. We saw considerable differences around the Bay, consistent with patterns of flowering, but also indicating that dispersal at large scales may be uncommon. In the Geelong arm, we saw genetically diverse meadows, with evidence of connections between different sites. Swan Bay was also diverse, but distinct from the Geelong sites. At the north of the Bay, we saw more reliance on asexual reproduction, with reduced genetic diversity. Some sites seem quite isolated, with limited sexual reproduction, low diversity, and few obvious connections to other seagrass beds. This pattern is particularly clear at Ricketts Point and Point Lonsdale. In the south east of the Bay, we saw diverse sites that seemed to have some connections to other seagrass areas. The genetic results do suggest that several parts of the bay are relatively discrete.

The most likely dispersal pathway is by drifting fragments. Seeds are not likely to disperse, as they sink, but we identified an unusual dispersive fragment that was produced by plants. These fragments remain healthy for up to 6 weeks, and may be an important means of recolonisation and genetic exchange. We found poor survival of these fragments in sediments, and remain uncertain about whether these fragments are produced in vast numbers and only a few ever establish, or if there are particular circumstances that allow them to become established. Seeds also are a paradox. Their large numbers ( $>10,000$  per  $m^2$ ) in some cases suggests an important ecological role, but there are questions about what causes them to germinate and how easily seedlings become established. We induced germination by changing salinity, but only achieved low germination rates. There are questions about whether there is a set of environmental cues that cause most seeds to germinate. A technique that involved high rates of fragment or seedling establishment would have potential for small-scale restoration or remediation projects.

The combined results from individual projects were used to develop a broad conceptual model for seagrasses in Port Phillip Bay. The conceptual model, illustrated using the Bellarine Bank, can be used to clarify expectations about “normal” seagrass dynamics around Port Phillip Bay and to highlight important drivers of seagrass. Identifying these drivers in a particular area allows identification of further work is needed, and is central to generating predictions of how seagrasses may respond to future Bay environments.

# 1 Introduction

Seagrasses are an important part of many coastal systems, but are also under threat in many areas around the world, as a result of a wide range of human activities, including habitat loss and changes to water quality. This is in part due their being unusual, as marine flowering plants that require considerable light. Many species have extensive structures below the sand and can form large clones. These clones can also be very long-lived. Others have shallow roots, grow quickly, and flower and seed often. These life cycle differences place individual seagrass species along a continuum from long-lived species that may be resistant to change, but poor at recovering through to those that may not persist, but readily colonize new areas. The dramatic losses of seagrasses elsewhere in the world make it important to understand the broad relationships between environmental variation (natural *and* anthropogenic) and seagrasses.

Resilience is the ability of a natural system to respond to external stressors without loss of essential function. We focus on the resilience of *Zostera* seagrasses in Port Phillip Bay because they are ecosystem “engineers”, whose presence has a dramatic influence on biodiversity and ecosystem function. Within the Bay, many species are found predominantly in seagrass meadows, and some, including important commercial and recreational species, depend on seagrasses for some part of their life cycle.

Mapping from aerial photography over at least 50 years has shown the most extensive areas of seagrass are in the southwestern quadrant of Port Phillip Bay (Figure 1). There are two *Zostera* species in the bay but the cover is dominated by *Zostera nigricaulis*. *Zostera muelleri* tends to be restricted to intertidal and very shallow subtidal habitats in Port Phillip Bay, whereas *Z. nigricaulis* is almost exclusively subtidal. Because it is not possible to tell the two species apart in aerial photography the two species are combined for mapping. The distribution of another seagrass species, *Amphibolis antarctica*, was not examined in this study because it accounts for less than 3% of the total seagrass area in Port Phillip Bay and is typical of more exposed locations along the open coast (Figure 1). There are areas of *Halophila* in some deeper areas. This latter seagrass does not form dense beds in the Bay nor is it thought to be an important engineer.

Historically, cover of *Z. nigricaulis* in Port Phillip Bay has varied greatly over long time scales, but this variation has not been consistent in different parts of the bay. For example, seagrass beds in the Blairgowrie and Point Richards areas showed a long term increase from the 1950’s to the late 1990’s but then dropped off dramatically. Seagrass in Swan Bay and at Kirk Point have shown quite different patterns over the same period. It is thought environmental variables, particularly nutrients and sediments, may be the drivers behind these fluctuations. Nutrients can have a positive effect when conditions are nutrient poor but a negative effect when levels are too high, promoting algal overgrowth. Sediments can adversely affect seagrass by blocking light in the water column or through movement of sand.

Seagrasses (and other organisms) may be resilient because they are able to resist external stressors, or because they have the capacity to sustain some losses, but then recover. Both of these aspects are important. When there is environmental change, resilience may also be modified. If that change makes some local habitats less suitable (e.g., because they become more turbid), resistance may be lowered. In a similar way, the ability to recovery from loss will be constrained by the current suitability of the habitat.

The seagrass resilience program addressed two main themes:

*What is the relationship between seagrasses and their physical environment, particularly sediments and nutrients?*

*How do seagrasses recover following loss?*

The focus of this work was Port Phillip Bay, which is not a uniform water body, but a large embayment with wide variation in physical processes, ranging, for example, from strong tidal currents and coarse sands in southern parts of the bay, to complex, wind-driven circulation and fine sediments

in the northern parts and up the Geelong Arm. Human activities are similarly variable, with higher population density around the northern half, catchment inputs at a few places, and the major influence of the Western Treatment Plant on the western shore. In this research program, we also sought to understand what this variation means for seagrasses, and, ultimately, for environmental managers:

*Are seagrasses within PPB well connected, or do they consist of isolated areas that may require separate management? How different are seagrasses in different parts of Port Phillip Bay?*

The overall results from this program will provide an information base for the Port Phillip Bay Environment management plan and contribute to the State of the Bays reporting, as well as making us better prepared to deal with future changes to the Bay.

## **1.1 Specific objectives and performance indicators**

### ***What is the relationship between seagrasses and their physical environment, particularly sediments and nutrients?***

Our aim was to identify the role nutrients and sediments play in Port Phillip Bay (PPB) at a range of spatial scales, and consider their role in driving past, current and future seagrass distribution and cover in PPB. This included examination of these processes at a broad, bay wide scale, followed by more targeted research conducted at regional and smaller spatial-scales.

Targeted research included field experiments on nutrient limitation, uptake and cycling, and turbidity and sedimentation effects on seagrass. Experiments were used to determine critical thresholds for nutrients, turbidity and sedimentation for seagrasses in Port Phillip Bay.

#### **Performance indicators**

1. The primary sources of nitrogen used by *Z. nigricaulis* in different parts of PPB are identified
2. The nutrient status of seagrasses in PPB, particularly in relation to nutrient limitation is mapped
3. When and how seagrasses utilise nutrients is understood through quantifying the uptake and flux of nutrients in seagrass beds
4. The effect that elevated nutrient and turbidity levels have on seagrass and epiphyte growth (including their relative importance) will be understood, including threshold levels that lead to significant change.
5. The relationship between large-scale hydrological (wind, waves, currents), physical (sediment type) and sediment transportation processes and seagrass distribution in PPB is understood
6. Sediment burial thresholds for *Zostera nigricaulis* seagrasses in PPB are determined

### **How do seagrasses recover following loss?**

When seagrass meadows decline, what are the main processes allowing them to recover? Are they replenished by growth of survivors, from seed banks in the sand, or do they rely on drifting seeds and seagrass fragments from nearby healthy populations?

We used a combination of field experiments and surveys in Port Phillip Bay and molecular genetics to answer these questions.

We described the resilience of seagrass beds, using field experiments to measure seagrass recovery rates and the contribution of seeds, local vegetative growth, and rafting, coupled with seasonal measurements of timing and level of seed production, the extent of rafting, and success of seeds and seagrass fragments. We also examined the changes to water quality that occur as seagrass beds shrink and grow.

#### **Performance Indicator**

7. Region-specific assessment of seagrass beds around PPB, detailing the roles of seeds, rafting fragments, clonal growth within beds and bed size and shape in resilience of beds.



## Are seagrasses within PPB well connected, or do they consist of isolated areas that may require separate management? How different are seagrasses in different parts of Port Phillip Bay?

We made this assessment using a combination of targeted work, in particular a range of genetic analyses that assessed the exchange of genetic material between different seagrass areas of the Bay, and results from other program components.

### Performance Indicator

8. An assessment of the extent to which local seagrass beds are discrete ecological units, compared to a reliance on a regional or a bay-wide network of seagrass areas. Part of this assessment was to include, if relevant, region-specific measures of seagrass recovery rates from disturbance.

### Synthesis

This work will be embedded in a conceptual model for seagrass habitat that will incorporate an understanding of the roles of nutrients and sediments in limiting seagrass growth, an understanding of resilience, and information on connectivity.

### Performance Indicator

9. A conceptual framework for seagrasses in PPB, incorporating regional and local water quality, sediment processes, and basic reproductive biology.

## 1.2 Technical details

The specific technical details of the work, including precise methods, data analysis, and more extensive results, are contained in the technical appendices referred to in section 5 of this report.

We used a wide range of innovative techniques across the individual projects, drawing on disciplines ranging from molecular genetics, through ecology, to geochemistry, oceanographic modelling, and GIS. Specific methods are described in the relevant technical appendices referred to in section 5 of this report.

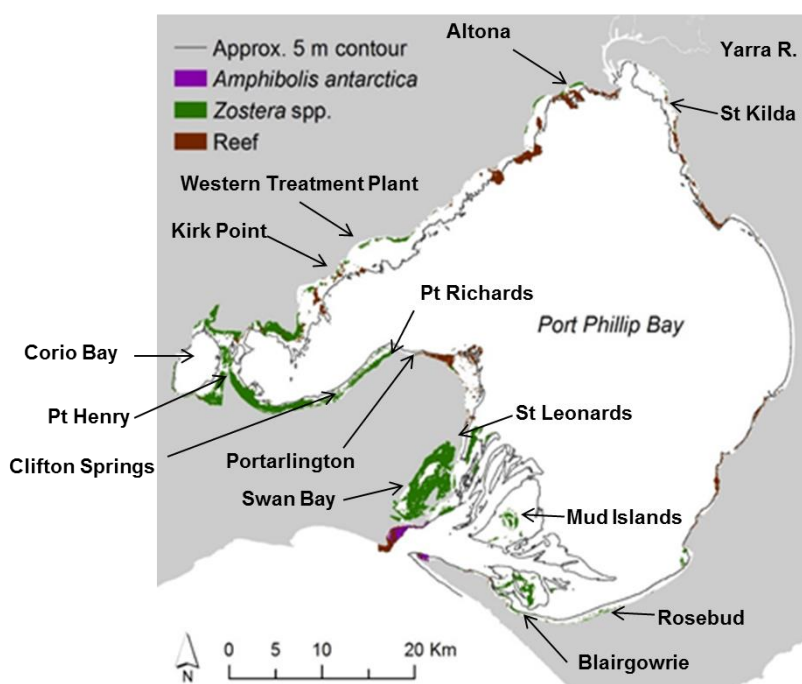


Figure 1 Seagrass distribution in Port Phillip Bay, 2000.

## 2 What are the important drivers?

### 2.1 Bay-wide processes

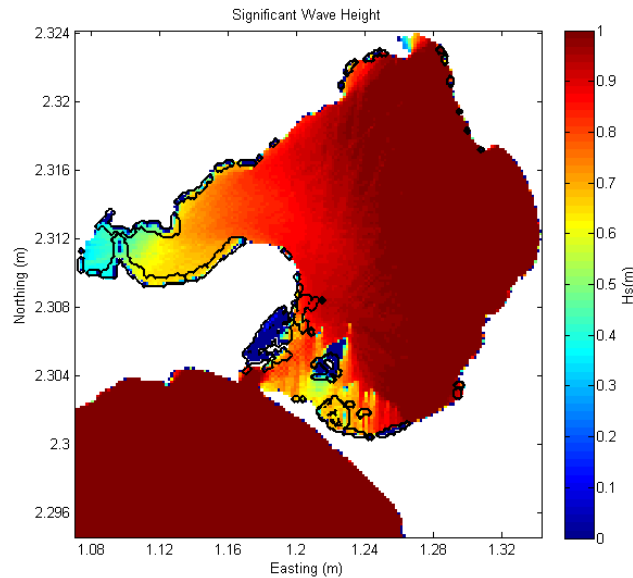
Historical seagrass mapping of Port Phillip Bay has shown that at the scale of the whole bay, the distribution of seagrass remains relatively constant, but there are large changes in seagrass cover over time in localised areas. We examined the links between bay-wide seagrass distribution and broad-scale hydrodynamic (waves, currents), physical (light, depth, salinity and temperature) and catchment (nutrient and suspended sediment levels) processes. This analysis was undertaken using a seagrass distribution map (surveyed in 2000) and hydrodynamic modelling undertaken in this program.

The bay-wide distribution of seagrass in Port Phillip Bay can be predicted from two physical processes: wave exposure and depth/light. Wave exposure excludes seagrasses from colonising wave-exposed coastlines, whilst depth/light determines the depth distribution of seagrasses in Port Phillip Bay through the availability of light for photosynthesis. This conclusion is consistent with other studies that have examined the environmental drivers of *Zostera* distribution at larger spatial scales. By comparison, there is less evidence that variation in water quality, represented by variables such as modelled total nitrogen, suspended solids or salinity, had a major influence on the distribution of seagrass across Port Phillip Bay on the bay-wide scale.

In Port Phillip Bay, the majority of seagrass is restricted to depths less than 6.0 m, where light at the bottom is greater than 30% of surface light. The deepest seagrass meadows in Port Phillip Bay mapped occurred at depths of 8 m and receive about 15% of surface light at the bottom at these depths. This figure is consistent with the maximum estimated depth of seagrass based on minimum light requirements of *Z. nigricalis*.

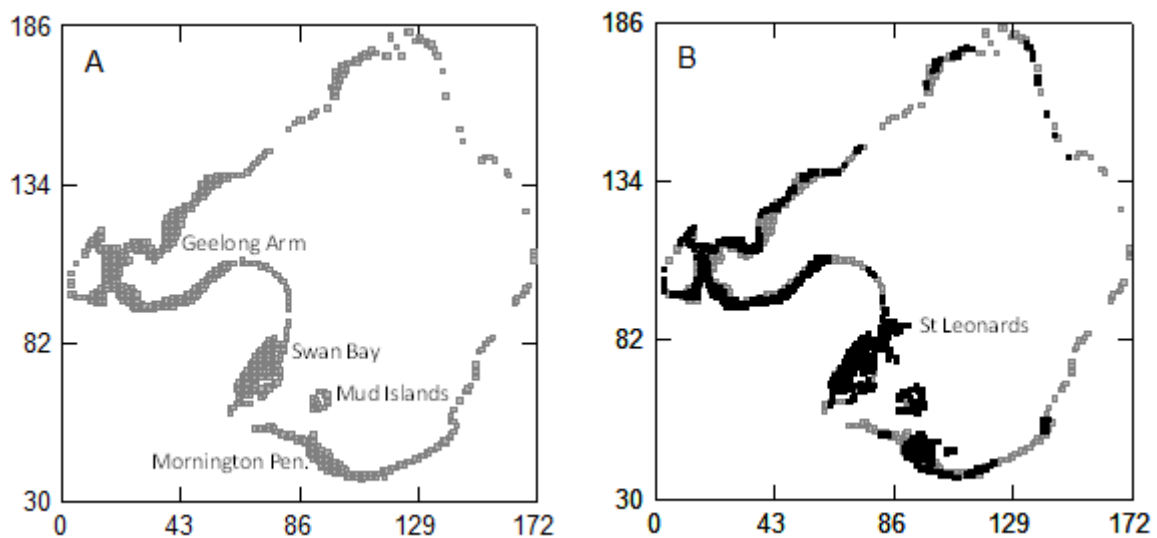
Although Port Phillip Bay contains significant areas of shallow bottom with sufficient light to support seagrass growth, the statistical analyses indicate that the distribution is limited by wave exposure. There appears to be a clear threshold where mean wave heights exceeded 0.38–0.43 m beyond which little seagrass occurs. In Port Phillip Bay the largest expanses of seagrass meadows are located in regions that are protected from the prevailing westerly winds, such as Swan Bay, Corio Bay and the western section of the Geelong Arm. In contrast, there is little seagrass along the eastern shore of Port Phillip Bay where the highest wave heights coincide with the longest fetch and exposure to prevailing wind direction within the bay. Turbulent wave action generated by winds may preclude shallow-rooted *Zostera* seagrasses from high energy environments through erosion, up-rooting and burial of seagrass plants, particularly during storm events. Wave turbulence also may keep seagrass populations in a constant state of re-colonisation at exposed locations.

In south-eastern Australia, *Z. nigricalis* is primarily restricted in distribution to marine embayments (such as Port Phillip Bay, Western Port and Corner Inlet) that provide protection from prevailing westerly wind-generated waves. However, the turbulent effect of waves are also significantly attenuated by depth and this may explain why *Z. nigricalis* can occupy deeper habitats in areas of higher wave exposure, such as deeper waters in the southern parts of Port Phillip Bay and Bass Strait where the turbulent effect of wave energy is substantially reduced at depth and light clarity is higher than for bays and inlets.



**Figure 2 Wave conditions modelled for the two year period of July 2009-2011, black outline indicates seagrass distribution**

Although depth/light and wave exposure are important factors, the seagrass habitat model for Port Phillip Bay only explains about 40% of the bay-wide seagrass distribution, with significant areas that that the model predicts are suitable for seagrass growth that do not have seagrass cover (Figure 3). This implies that in regional areas of Port Phillip Bay there are other factors at play that influence the cover of seagrass.



**Figure 3 Predicted seagrass habitat (A) comprising grid cells with mean wave height < 0.38 m and bottom light > 33% surface light, and (B) with grid cells containing seagrass (black) overlaid.**

## 2.2 Regional processes

There are major regional fluctuations in seagrass cover over time. For example, in regional areas such as Blairgowrie, St Leonards and Bellarine Bank, there has been a long term increase in seagrass cover from the 1950's to the late 1990's but then a dramatic decline in the 2000's. The patterns in other regions, such as Kirk Point and Swan Bay, however, were quite different.

We hypothesised that the most likely drivers of these regional fluctuations were nutrients, suspended sediments and sediment movement. Nutrients are primarily introduced from the catchment and increasing levels can promote seagrass growth but if levels are too high can cause algal blooms that block light reaching seagrass. Similar to high levels of nutrients, suspended sediments introduced from the catchment can increase turbidity and reduce light reaching seagrass. Finally, movement of sediment within the bay caused by currents and waves can lead to regional smothering of seagrass beds.

## Nutrients

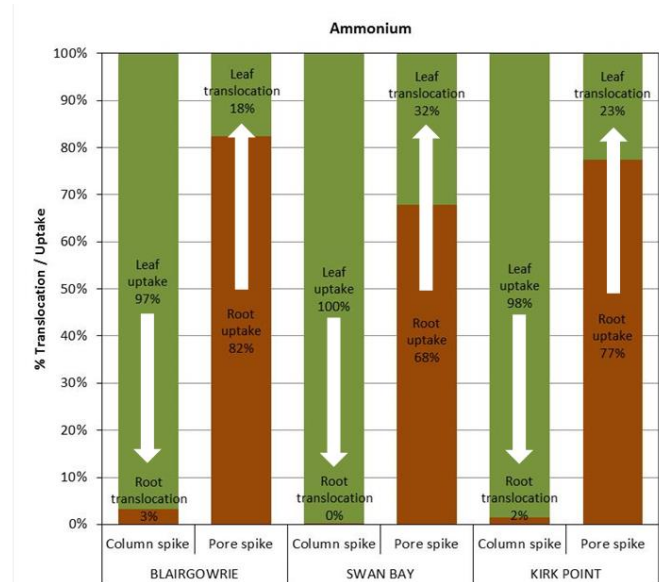
### How do seagrasses in Port Phillip Bay obtain their nutrients?

In Port Phillip Bay, seagrasses obtain nitrogen from a number of sources in what is generally a nutrient poor environment. Dissolved nitrogen enters the bay from the catchment and Western Treatment Plant (WTP) and also occurs in groundwater beneath the sediments of the bay. At three sites, chambers (Figure 4) were placed on seagrass plants and stable isotope ( $^{15}\text{N}$ ) labelled nitrogen (ammonium and nitrate) was introduced to the chamber. These experiments showed that *Z. nigricaulis* can take up dissolved nutrients both from the above (leaves) and below (roots and rhizome) ground parts of the plant. Nitrogen can also be transported (translocated) within the plant between above and below the sediment, although the dominant pathway is from the roots/rhizomes to the leaves (Figure 5). The results showed that *Z. nigricaulis* has a clear preference for ammonium over nitrate as the source of nitrogen. This result is comparable to other species of seagrasses, as the plant requires far less energy to transform the reduced source of inorganic nitrogen (ammonium) into organic nitrogen than the oxidised forms (nitrate or nitrite).

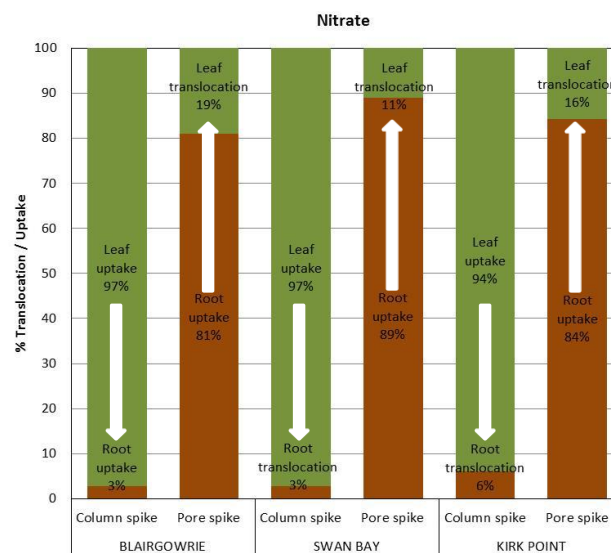


**Figure 4 Benthic chambers used for experiments on nutrient uptake and translocation**

a)

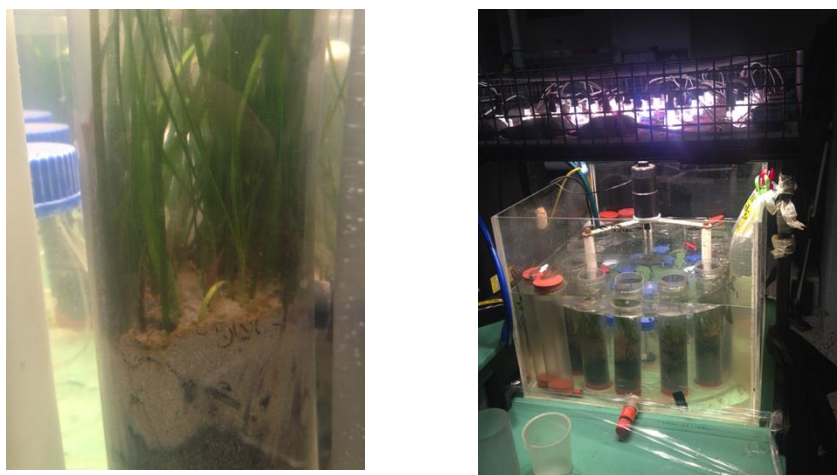


b)



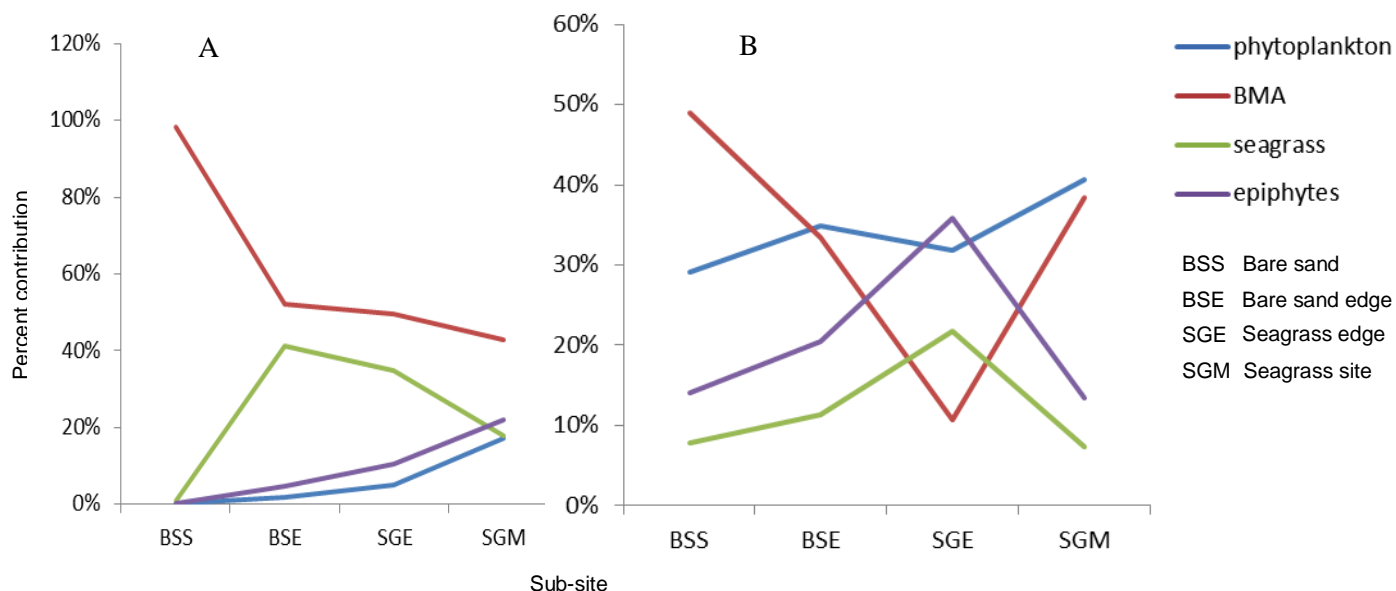
**Figure 5 Translocation and uptake of (a) ammonium and (b) nitrate by leaves and roots of *Zostera nigricaulis* at Blairgowrie, Swan Bay and Kirk Point in Port Phillip Bay. Percentage translocation is the amount of ammonium or nitrate translocated to the leaves or roots, calculated as a percentage of the total ammonium or nitrate assimilated by the seagrass. The arrows in the figure depict the movement of ammonium or nitrate from one compartment to the other, and nutrients were added to the water column or added to pore water in the sediments.**

In addition to dissolved forms of nitrogen in the water column or pore water, seagrasses can utilise gaseous nitrogen by way of nitrogen fixing bacteria. Some of the highest nitrogen fixation rates measured in sediments are associated with seagrass, making these habitats a potentially important source of nitrogen to nitrogen limited coastal ecosystems. Cores of seagrass and sediment were taken from 9 sites around Port Phillip Bay and the level of nitrogen fixation was determined in the laboratory. Levels of nitrogen fixation were relatively low and ranged up to approximately 15% of the plants' requirements. Although only a small proportion of the plants' needs, these levels are higher than recorded in other temperate areas (mainly Europe) and may reflect the low nutrient status of Port Phillip Bay. Nitrogen fixation rates in general were significantly related to seagrass biomass, supporting the hypothesis that seagrass beds can be the source of new nitrogen inputs to coastal ecosystems.



**Figure 6 Experimental set up used to measure N<sub>2</sub> fixation rates.**

An important pathway for seagrass to obtain nutrients is through the breakdown of organic material at the sediment surface, introducing a pool of ammonia to the porewater that can be taken up by the seagrass. Although some of this comes from internal re-cycling of dead seagrass material, other organic material such as phytoplankton, epiphytic algae that grows on seagrass and single-cell algae that live on the sediment (BMA: benthic microalgae) can contribute this pool of organic material. In fact, because seagrass beds have the effect of reducing waves and currents in the area, they can become “particle traps” where suspended organic material is deposited and can break down releasing nutrients. We conducted a study on this process in seagrass beds at Point Richards and Clifton Springs. Currents at the bottom were 25 – 33 % lower inside seagrass beds than nearby bare sand areas. Stable isotopes are forms of an element with more (heavier) or less (lighter) neutrons relative to protons. Ratios of these forms are very useful for identifying plant sources. In our study, stable isotope ratios of carbon and nitrogen were used to identify the plant sources of particulate organic carbon (plant detritus) within and outside the seagrass beds. The highest contributors within the seagrass beds were BMA at Clifton Springs, and phytoplankton and BMA at Point Richards (Figure 7). These results suggest that seagrass beds are gaining nitrogen from the breakdown of plant detritus they trap from the water column. The relative importance of particle trapping versus internal recycling of seagrass detritus to seagrass nutrient uptake is likely to vary around the bay depending on factors such as nutrient levels and current/wave conditions.

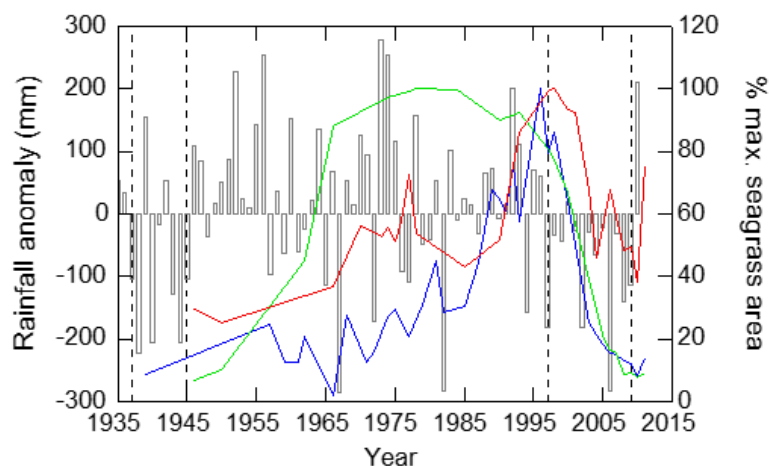


**Figure 7 Percent contributions of benthic microalgae (BMA), phytoplankton, seagrass and algal epiphytes to plant detritus in the sediment at A) Clifton Springs and B) Point Richards**



## Regional differences in the sources of nutrients used by seagrass in Port Phillip Bay and the potential for nutrient limitation

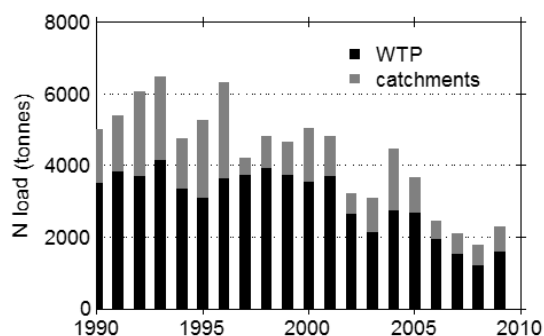
Historical trends in seagrass cover in the Bay indicate that nutrient availability may influence the regional cover of seagrass. Historical time series from aerial photography show that seagrass area at three locations in the southern and central part of the bay increased from 1960 to the mid-1990s and then declined rapidly from the late 1990s onwards. This decline coincided with a prolonged period of drought in southern Australia (1997–2009), characterised by lower freshwater and nutrient inputs into the bay. Over a longer historical period, peaks and troughs in seagrass area coincided with respectively wetter and drier periods in Victoria's climate (Figure 8). This has led us to hypothesise that the patterns of seagrass expansion and decline in PPB may be linked to nutrient availability.



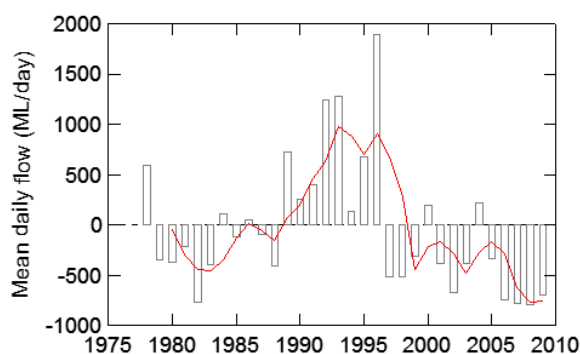
**Figure 8** Mean annual rainfall anomaly for Victoria 1935–2012 with changes in % maximum seagrass cover at three locations: Blairgowrie (blue line), St Leonards (red line) and Bellarine Bank (green line) overlaid. Dashed lines represent WWII drought (1937–1945) and millennium drought (1997–2009).

Seagrasses potentially acquire nitrogen (N) from a range of sources within PPB. The largest source of N into PPB originates from the Western Treatment Plant (WTP), followed by inputs from the Yarra River catchment, smaller rivers and creeks, atmospheric inputs (deposition of nitrogen from the atmosphere that dissolves and N-fixation of nitrogen gas) and seasonal influxes from Bass Strait.

The WTP and catchments contribute over 85% of the total N load entering PPB, although in both cases the overall contribution from these sources declined during the drought (Figure 9). Southern Australia experienced the longest and most severe drought on record from 1997–2009. Freshwater river flows from the Yarra River declined substantially from 1997 onwards (Figure 9) and this coincided with reduced nitrogen loads to the bay (Figure 10).

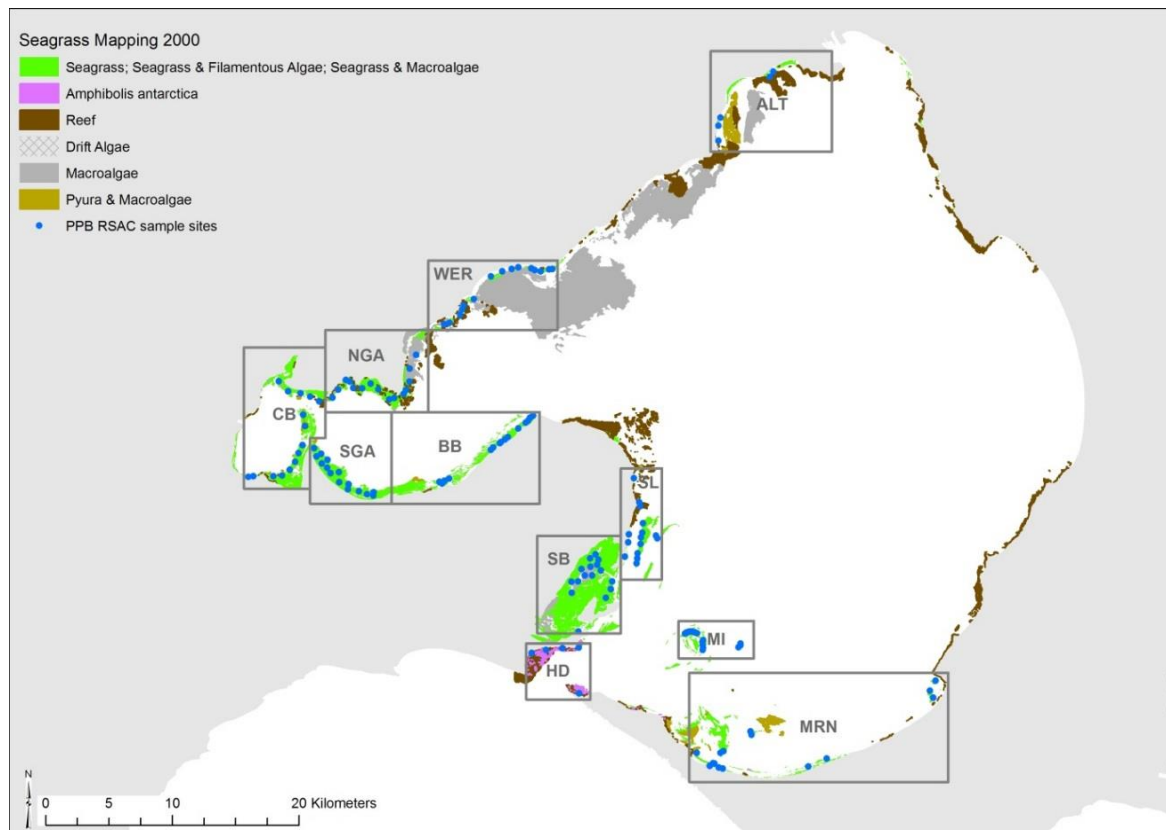


**Figure 9** Annual total N contribution to Port Phillip Bay from WTP and catchments from 1990–2009. Source: Melbourne Water and EPA E2Port catchment model



**Figure 10** Mean annual daily flow in relation to the average flow for the Yarra River 1978–2009 showing decline in 3-year moving average (red) during drought (1997–2009).

The importance of N from different sources to seagrass growth is also likely to vary spatially, reflecting the proximity of seagrass beds to different sources, and the role that hydrodynamic processes play in dispersing N in the water column and via food webs. Accordingly, there is a need to identify the sources of N utilised by seagrasses in order to understand how changes in the availability of nutrients from different sources may influence seagrass growth in different parts of PPB. We estimated the contribution of different sources of N to seagrass growth in 11 regions where seagrass predominantly occurs in PPB (Figure 11), using stable isotopes of N.

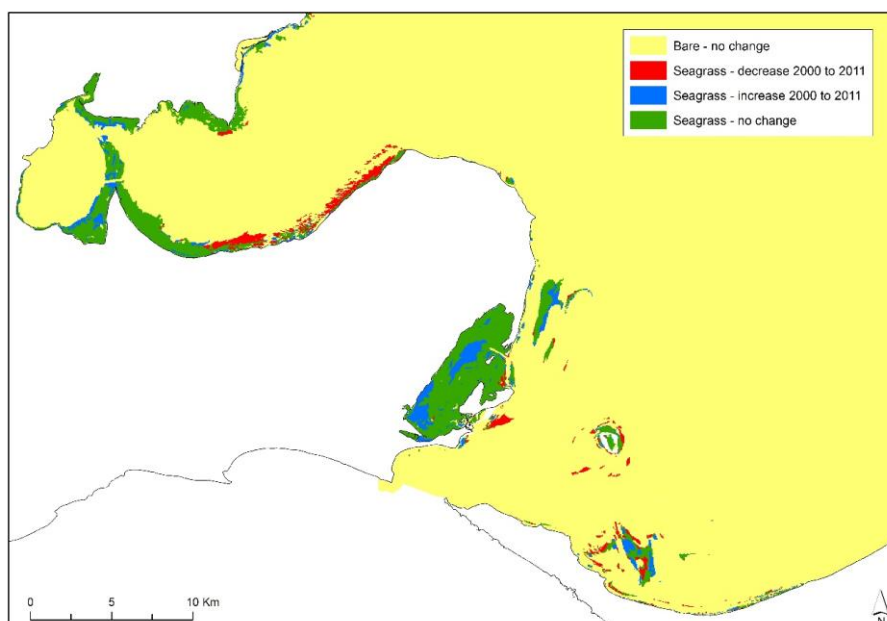


**Figure 11 Map of PPB showing seagrass regions and sites sampled in this study. Regions include ALT: Altona, WER: Werribee, NGA: North Geelong Arm, CB: Corio Bay, SGA: South Geelong Arm, BB: Bellarine Bank SL: St Leonards, SB: Swan Bay, HD: PPB Heads, MI: Mud Islands, and MRN: Mornington Peninsula.**

Stable isotopes are powerful tools for linking nitrogen in marine ecosystems to terrestrial and other sources. Sources of N have signatures that are retained and reflected in the ratio of elemental isotopes present (i.e.  $^{15}\text{N}/^{14}\text{N}$  or  $\delta^{15}\text{N}$ ). The contribution of different N sources can be inferred by comparing signatures in seagrass tissues in different parts of Port Phillip Bay to those of the sources using IsoSource mixture modelling.

We also examined whether regions where seagrass declined during the drought were dependent on N from the catchment and whether seagrass from these regions displayed evidence of N-limitation. Analysis of aerial photography revealed that seagrass area declined along the Bellarine Bank, Mornington Peninsula coast and around Mud Islands between 2000 and 2011 during the drought (Figure 12). By comparison seagrass areas in Swan Bay, Corio Bay and the western section of the Geelong Arm either remained unchanged or increased during this period.

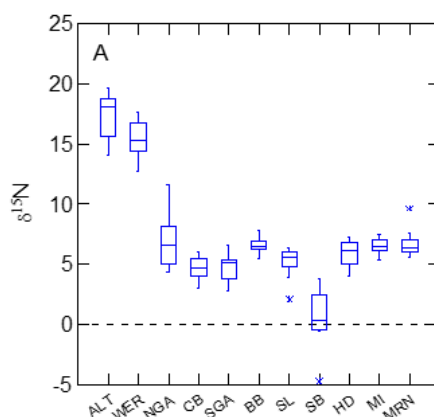




**Figure 12 Change in seagrass area between 2000 and 2011 in southern and western Port Phillip Bay.**

At a large spatial scale  $\delta^{15}\text{N}$  signatures in seagrass tissues displayed a clear spatial pattern reflecting the regional importance of a range of nitrogen (N) sources in Port Phillip Bay. The  $\delta^{15}\text{N}$  signatures of seagrass tissues fell into three broad categories: highly enriched (13–20 ‰  $\delta^{15}\text{N}$ ), depleted (-1–5 ‰  $\delta^{15}\text{N}$ ), and background levels typical of most of the bay (5–8 ‰  $\delta^{15}\text{N}$ ) (Figure 13).

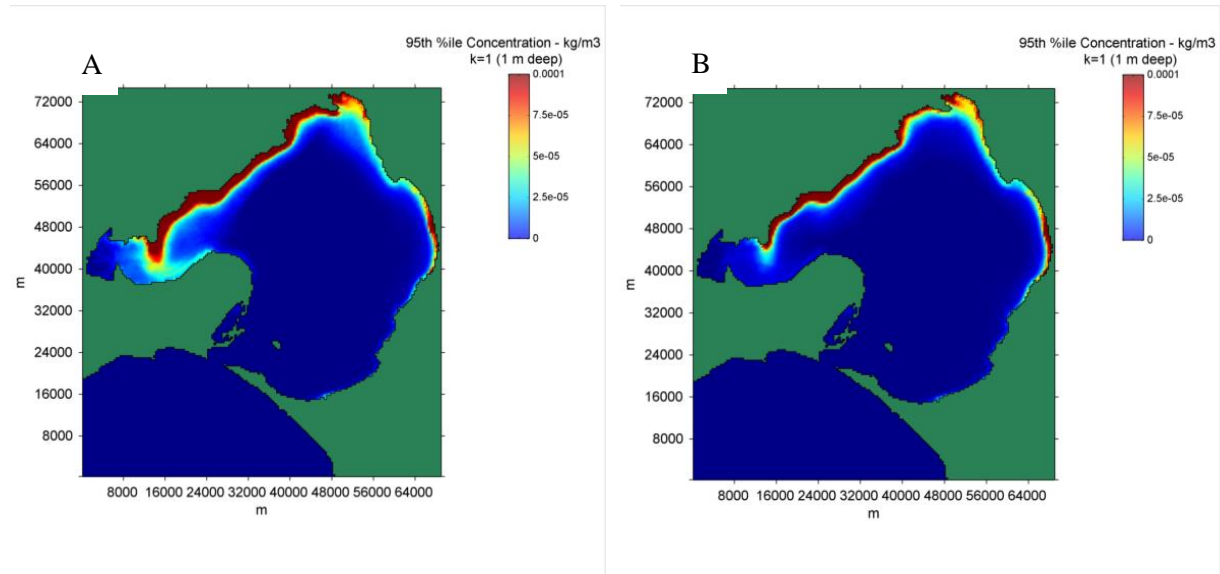
Seagrass tissues sampled along the northern coast of Port Phillip Bay (ALT) and adjacent to WTP (WER) were highly enriched in  $\delta^{15}\text{N}$ . This signature is characteristic of sewage derived N originating from the WTP. This distinctive signature means that the importance of this source for seagrass growth in Port Phillip Bay can be readily traced by measuring  $\delta^{15}\text{N}$  in seagrass tissues.



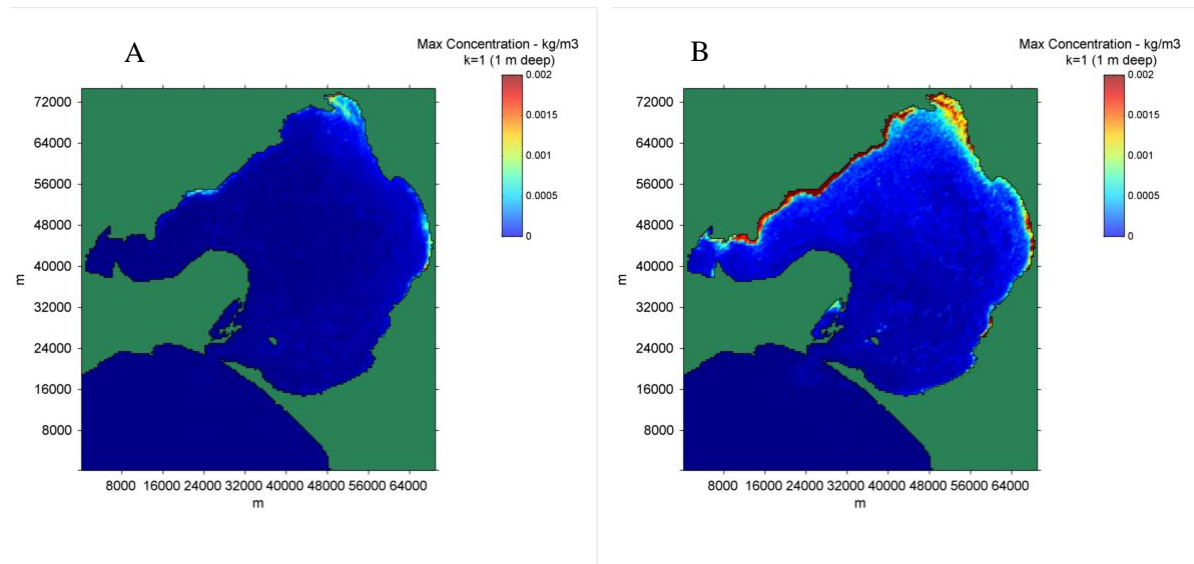
**Figure 13 Boxplot of stable isotope signatures of  $\delta^{15}\text{N}$  for leaf tissues collected from 11 regions across PPB.**

Our modelling studies showed that nitrogen released by the WTP is primarily retained along the north-western shore of the bay. The dispersal of Total Nitrogen from the catchment and Western Treatment Plant (WTP) for the periods 1999-2000 (dry) and 2010-2011 (wet) was simulated with the hydrodynamic and catchment models (Figure 14). The most notable pattern is that nitrogen levels from catchment/WTP inputs are only elevated along the north-west and north-east coasts of the bay (Figure 14). Although seagrass is found in areas of relatively high nutrient status (Altona and the Werribee area) associated with major inflows from the WTP and Yarra, as well as areas of relatively

low nutrient status (Bellarine Bank, St Leonards), the latter regions appear to have more continuous stands, whereas those closer to the large inflows are more fragmented. There is also a clear difference in the reduction in outflow from the WTP between the two time periods (as a result of ammonia reduction works that commenced in 2004) (Figure 14). When the WTP inflows are removed from the simulation (Figure 15), the major effect of the ~50% increase in total load from the catchment during the July 2009-2011 period can be seen affecting the north-west and north-east coasts.



**Figure 14 Total Nitrogen shown as the 95 percentile concentrations for (A) 1999-2000 and (B) 2010-2011**



**Figure 15 Total Nitrogen results shown as maximum concentration experienced during (A) 1999-2000 and (B) 2010-2011, for catchment inflows only (No WTP).**

The highly enriched  $\delta^{15}\text{N}$  signatures found along the northern coast of PPB are countered by seagrass tissues at a number of locations with highly depleted  $\delta^{15}\text{N}$  signatures (Figure 13). Depleted  $\delta^{15}\text{N}$  signatures were characteristic of seagrass tissues sampled from Swan Bay, but also parts of Corio Bay and the southern Geelong Arm. These protected locations support dense, extensive seagrass beds dominated by organically-rich fine sediments. Low  $\delta^{15}\text{N}$  values have typically been associated with plants that derive a high proportion of their N from compounds fixed directly from the atmosphere. However, in Port Phillip Bay direct measurement has shown that N-fixation is only a minor source of N for seagrasses (i.e. <15% of total N required) and that the  $\delta^{15}\text{N}$  signature of leaf tissues was unrelated to the contribution provided by nitrogen fixation. We attribute the source of the depleted

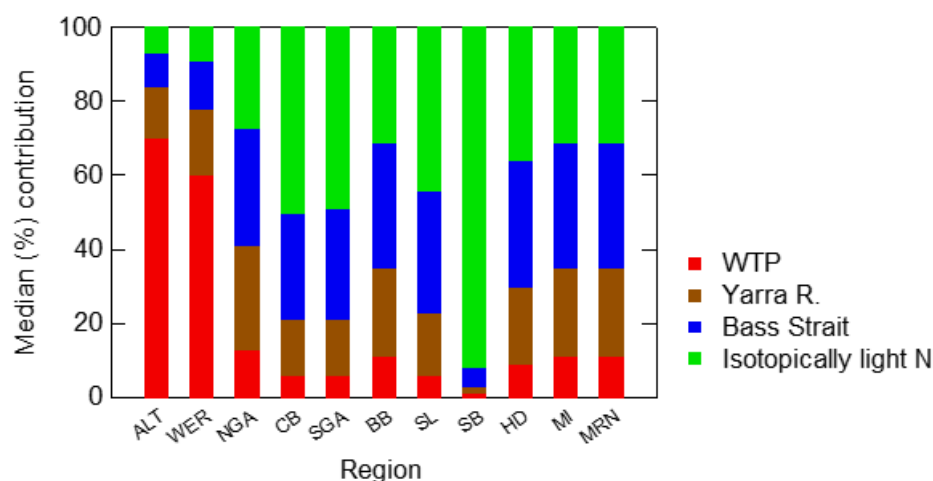
$\delta^{15}\text{N}$  signatures of seagrasses to the lighter nitrogen  $^{14}\text{N}$  that is produced when bacteria break down organic matter in the sediment. This conversion to the lighter N isotope by bacterial activity is an example of a process known as fractionation. The presence of low  $\delta^{15}\text{N}$  ratios in seagrass tissues therefore reflects the high availability of  $^{14}\text{N}$  in sediments, leading to uptake by *Z. nigricaulis* roots of the lighter isotope  $^{14}\text{N}$  relative to  $^{15}\text{N}$ .

The majority of seagrass surveyed in Port Phillip Bay had  $\delta^{15}\text{N}$  signatures in the range of 5–8 ‰ units. This includes seagrasses from the Mornington Peninsula, Mud Islands, St Leonards and Bellarine Bank regions. Seagrasses in the southern part of PPB are largely geographically removed from the spatial influence of the WTP by the dominant circulatory currents in the bay and this conclusion is consistent with the absence of a distinctive ‘WTP’ signature in the tissues of seagrass beyond the north coast of PPB. The shape and extent of the Yarra plume is highly dependent on river flows and wind speed and direction. During periods of high river flows and westerly winds the Yarra plume is directed along the eastern shore of PPB delivering N to the eastern and southern regions of the bay. Southern PPB also receives seasonal (autumn-winter) influxes of nitrate from Bass Strait delivered by tidal exchange with Bass Strait. Although the overall size of this source is small in comparison to the WTP and catchments, its relative importance to seagrasses growing in southern part of PPB may be high.

A number of general statements can be made about the relative importance of N sources utilised by seagrasses based on the results of the mixing model analysis (Figure 16). First, the WTP was the major source of N utilised by seagrasses in the ALT and WER regions, but a relatively minor source for all other regions. The importance of this source is therefore largely restricted to the north coast of PPB, where the prevailing local currents limit the dispersal of N to the coastline adjacent to the WTP. Second, fractionation of recycled N in sediment porewater was the major source of N utilised by seagrasses in Swan Bay and is also likely to be an important source in other protected locations in PPB where sediments are dominated by fine, organically-rich sediments. Finally, seagrasses in all other regions were dependent on a range of sources, rather than any single source. For seagrasses in these regions contributions from the catchment, Bass Strait and fractionation of N within the sediments were all important N sources.

### **Is there a link between the seagrass declines and sources of N utilised by seagrasses in Port Phillip Bay?**

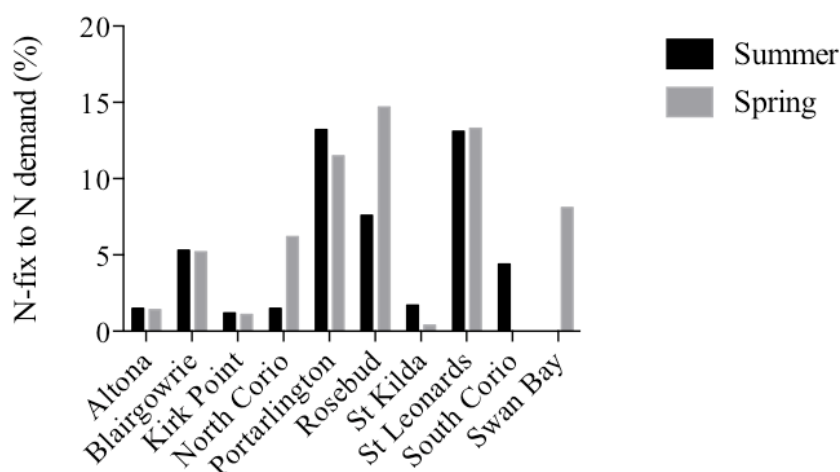
Regional patterns of N source utilisation did correspond with changes in seagrass extent between 2000 and 2011. *Z. nigricaulis* in regions where seagrass declined between 2000 and 2011, including the Bellarine Bank (BB), Mornington coast (MRN) and Mud Islands (MI), used a similar array of sources including a significant contribution from the Yarra catchment (Figure 16). By comparison, *Z. nigricaulis* in regions where seagrass area remained relatively stable between 2000 and 2011, such as Swan Bay (SB), Corio Bay (CB) and the southern Geelong Arm (SGA), were largely dependent on a single source, fractionation of porewater N, and are much less dependent on N originating from the catchment. These regions tend to be isolated from both major sources, both in terms of distance from source, but also by the way in which nutrients are dispersed and circulated within the bay.



**Figure 16 Percentage contribution of Nitrogen from four sources to regional areas of Port Phillip Bay: WTP, Yarra R., Bass Strait and isotopically light N in each region.**

It is counterintuitive that dependence on a single source may lead to greater resilience and stability in terms of seagrass area within Port Phillip Bay. However, this will depend greatly on which N sources are utilised and how variable these sources are through time. We know that N inputs from the catchment and the WTP declined significantly during the drought (Figure 5) but know less about the variability of the other N sources considered in this study: N from Bass Strait and fractionation of porewater N within the sediments. It is therefore conceivable that reductions in N from the catchment may have contributed to the decline of seagrasses at a number of locations in Port Phillip Bay because these locations coincide with regions where N from Yarra River is likely to be an important source.

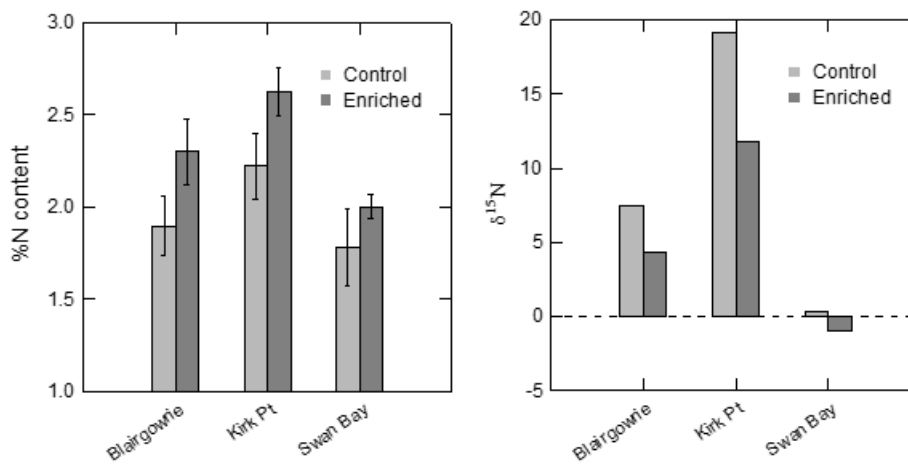
We examined whether there was evidence of nutrient-limitation in regions dependent on catchment sources of N. One source of evidence came from the experiments on Nitrogen fixation. The importance of Nitrogen fixation as a proportion of Nitrogen demand would be expected to increase in regions where Nitrogen was limiting. The results support the idea that areas influenced by the catchment are Nitrogen limited, with highest N fixation in relation to N demand occurring at Portarlington, Rosebud and St Leonards (Figure 17).



**Figure 17 The proportion of nitrogen demand derived from nitrogen fixation at 10 study sites across Port Phillip Bay.**

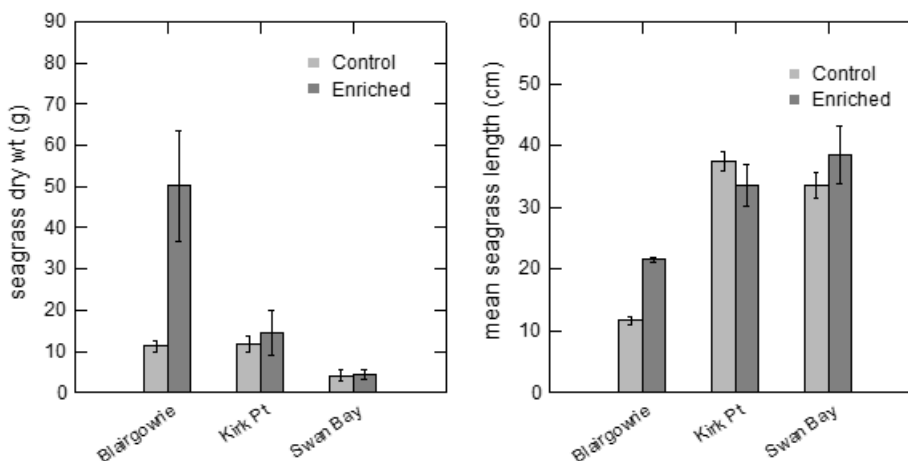
Evidence of N-limitation was further explored using experiments in which the sediments were enriched with nutrients. A number of studies have shown that seagrasses take up a large proportion of their nutrients from the sediments via the root system, and in Port Phillip Bay concentrations of P and

N are up to 10 and 100 times greater in the sediment porewater than in the water column. Sediment nutrient levels were experimentally enriched at three sites indicative of the importance of different sources of N for seagrass growth: Swan Bay (0‰  $\delta^{15}\text{N}$  in seagrass tissue), Blairgowrie (7‰) and Kirk Point (17‰). Stable isotopes of N indicate that seagrasses at Swan Bay primarily use isotopically light N in the porewater, Kirk Point primarily N from the WTP including some N taken up through the leaf tissues, and Blairgowrie from a range of N sources including the catchment. Enriching the sediments experimentally with slow release fertiliser (used to realistically simulate normal input from the catchment rather than a pulsed event) led to a significant increase in the level of Ammonia in the sediment porewater by between 20 and 500 times depending on the location and ambient  $\text{NH}_3$  levels. Moreover, seagrass tissues in enriched experimental treatments at Blairgowrie and Kirk Point had higher %N content and lower  $\delta^{15}\text{N}$  signatures than control treatments (Figure 18). Indicating that seagrass at these sites had assimilated N compounds (released by the fertilizer) with a lower  $\delta^{15}\text{N}$  signature (i.e.  $\delta^{15}\text{N} = 2$ ).



**Figure 18 Mean ( $\pm$  se) % N content and mean  $\delta^{15}\text{N}$  (‰) in leaf tissue collected from control and enriched treatments at Blairgowrie, Kirk Point and Swan Bay after 5 months.**

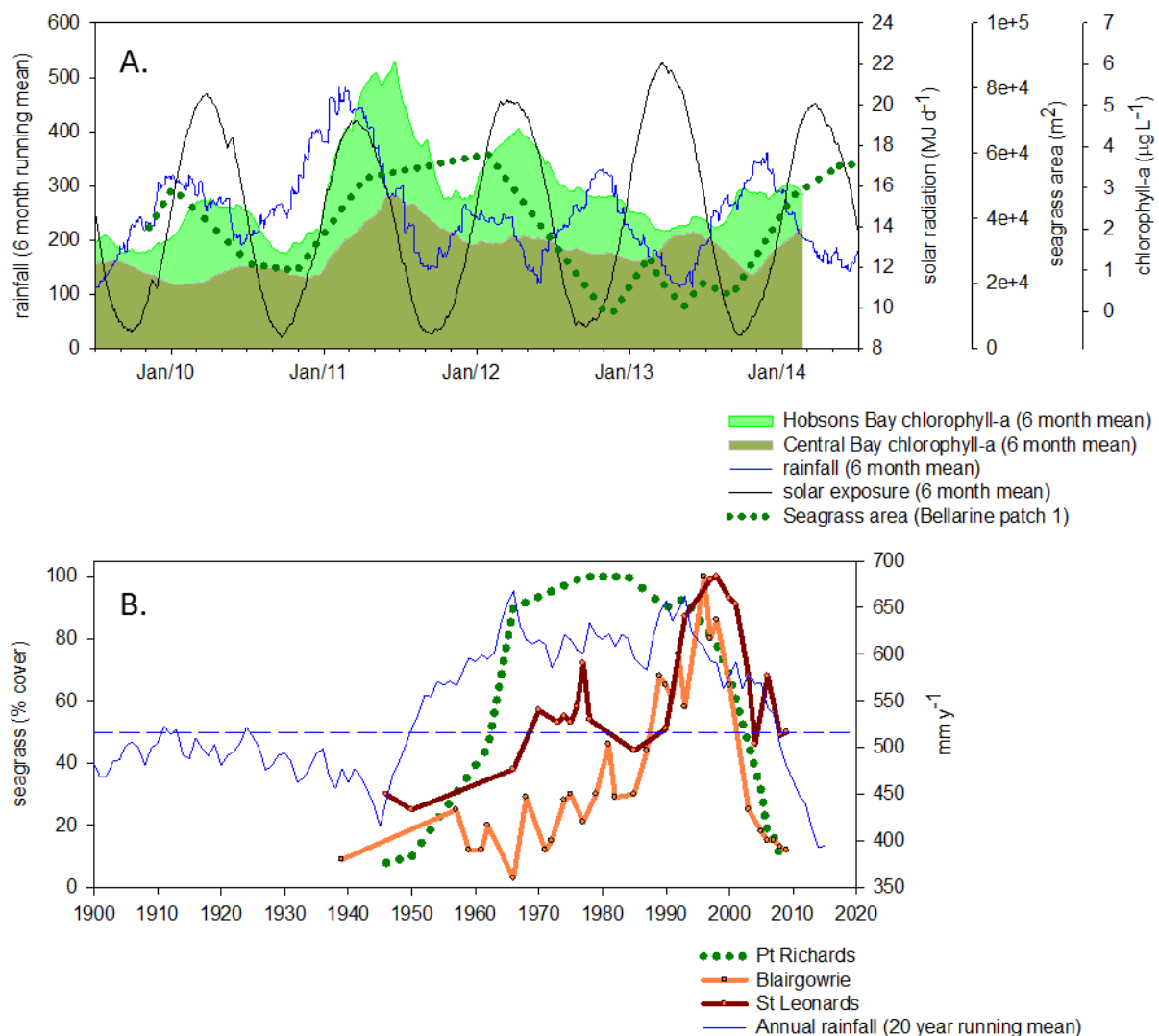
Seagrasses only showed evidence of N-limitation at the Blairgowrie experimental site (Figure 19). After 5 months, seagrass biomass was 340% greater, and leaf length 80% greater in the enriched compared to the control experimental treatment at the Blairgowrie site. This implies that seagrass growth at this location is limited by a lack of nutrients (i.e. growth is substantially enhanced following the addition of nutrients). No enrichment effect was detected at either Swan Bay or Kirk Point.



**Figure 19 Mean seagrass dry weight and mean length for control and enriched treatments at Blairgowrie, Kirk Point and Swan Bay after 5 months.**

Nutrients may not only be a controlling factor for long-term changes in seagrass in catchment dependent areas, but also for short-term, inter-annual variation. We undertook a detailed examination of seagrass cover over five years on the Bellarine Bank from 2009 to 2014. There was a general pattern where both phytoplankton and seagrass cover tracked increased rainfall, particularly after the rainfall that occurred in the spring growing season in 2010 (Figure 20). Given the ability of seagrass meadows along the Bellarine Banks to use the trapping of organic particles, we hypothesise that the increase in phytoplankton resulted in significant nutrient inputs to the Bellarine Banks seagrass system at this time resulting in the expansion of meadows.

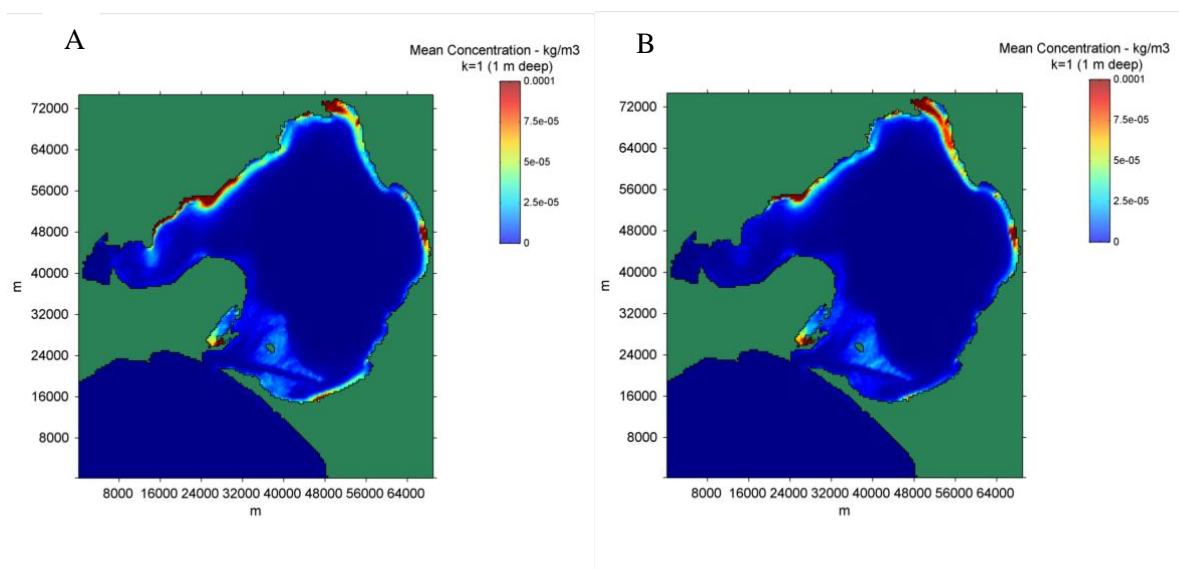
The large increase in coverage between 1940 and 1980 (which represented a 50 fold greater increase than observed during 2010 – 2011) occurred during a period of sustained above-average rainfall (Figure 20). Greater nutrient loadings during this time most likely caused an overall nutrient enrichment of the bay system relative to extended dry periods, leading to greater productivity of phytoplankton and dependent seagrass meadows along the Bellarine Banks. Meadows at Blairgowrie and St Leonards are situated in regions that benefit less from bay phytoplankton productivity due to prevailing currents, hence the delayed and muted response in seagrass coverage to the higher rainfall period. The rapid decline in seagrass since the onset of the millennium drought is consistent with a decline in nutrient loadings to the bay, but it may also reflect changes in other pressures such as prevailing winds and bay circulation patterns.



**Figure 20 A. Inter-annual variability in seagrass coverage at Bellarine Banks and the 6 month running means for light, rainfall, and chlorophyll-a. B. Long period trends in seagrass coverage in Port Phillip Bay and the 20 year running mean for annual rainfall.**

### Light & turbidity

Bay-wide scale modelling on the 400m grid provides a general overview of the potential for light reduction that can constrain seagrass growth. Modelled conditions for total suspended solids (TSS) are shown in Figure 21 and show that seagrass habitats experience a range of concentrations. The results for the northwest coast, including Altona-Werribee seagrass regions, indicate there is a potential for depth limitation based on reduced light from TSS. The TSS results show decreased loading from the WTP in the later period (after the ammonia upgrade) along the northwest coast, but significant levels were present in both periods (Figure 21). In contrast, areas such as Corio Bay and the southern Geelong Arm remained low throughout (Figure 21).



**Figure 21 Total suspended sediment (TSS) results shown as mean concentration experienced during A) 1999-2000 (dry) and B) 2010-2011(wet).**

There is a positive relationship between light attenuation ( $K_d$ ) and suspended sediments (TSS) around the bay. Light levels are significantly reduced in the northwest of the bay with only 6% of light reaching 5m depth. This may be compared with a minimum value of 10-15% required by *Z. nigricaulis* for growth and survival. This area is characterised by fine sediments and light attenuation ( $K_d$ ) values of are frequently  $\sim 0.45$ , whereas background values for the bay are typically 0.2-0.25. Overall these results may help explain why seagrass cover along this coast is lower than would be predicted based on depth and wave exposure (Figure 3Figure 6).

We looked in more detail at the effect of light limitation on seagrass habitats of the north-west coast of Port Philip Bay by running a fine-scale 50 m grid model of the Geelong Arm. This finer scale model considers additional factors affecting light such as phytoplankton, bottom topography and resuspension of sediments. The finer scale modelling simulates the mobilization of the sand, silt and clay fractions from both the catchment inflows and the resident sediments on the sea bed, to provide a more accurate representation of light clarity and sediment movement. Finer resolution of coastal topography and varying bed friction is also accounted for to identify accumulation and erosion zones for both settled and suspended sediments.

In areas where finer sediments dominate such as the northwest coast of Port Phillip Bay, wave induced shear stress on the bottom is required to exceed thresholds to re-suspend the fine sediment. Figure 22 shows the resultant silt fraction associated with suspended material in the Geelong Arm, related to sediment size and wave stress. The results indicate that resuspended sediments have the greatest impact on light in the Werribee seagrass region, while once again resuspension of fine sediments is very low in Corio Bay and the southern Geelong Arm (Figure 22).

Our studies also investigated the characteristics of turbidity at the very fine scale of individual seagrass beds. At Clifton Springs the turbidity was reduced over a large seagrass patch (Figure 23) consistent with previous studies on turbidity around seagrass beds. This lower turbidity may have been related to particle trapping or reduced re-suspension of sediments under the lower wave and current conditions experienced in the seagrass bed. Other results, however, suggested that this pattern may not be consistent for all seagrass sites, depending on the local environment.



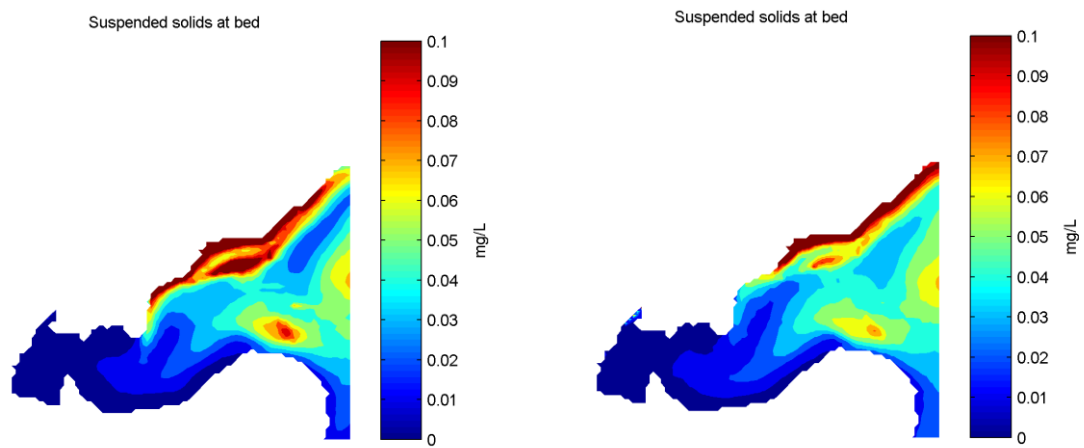


Figure 22 Silt suspended (95%ile) at bed for 1999-2000 and 2010-2011

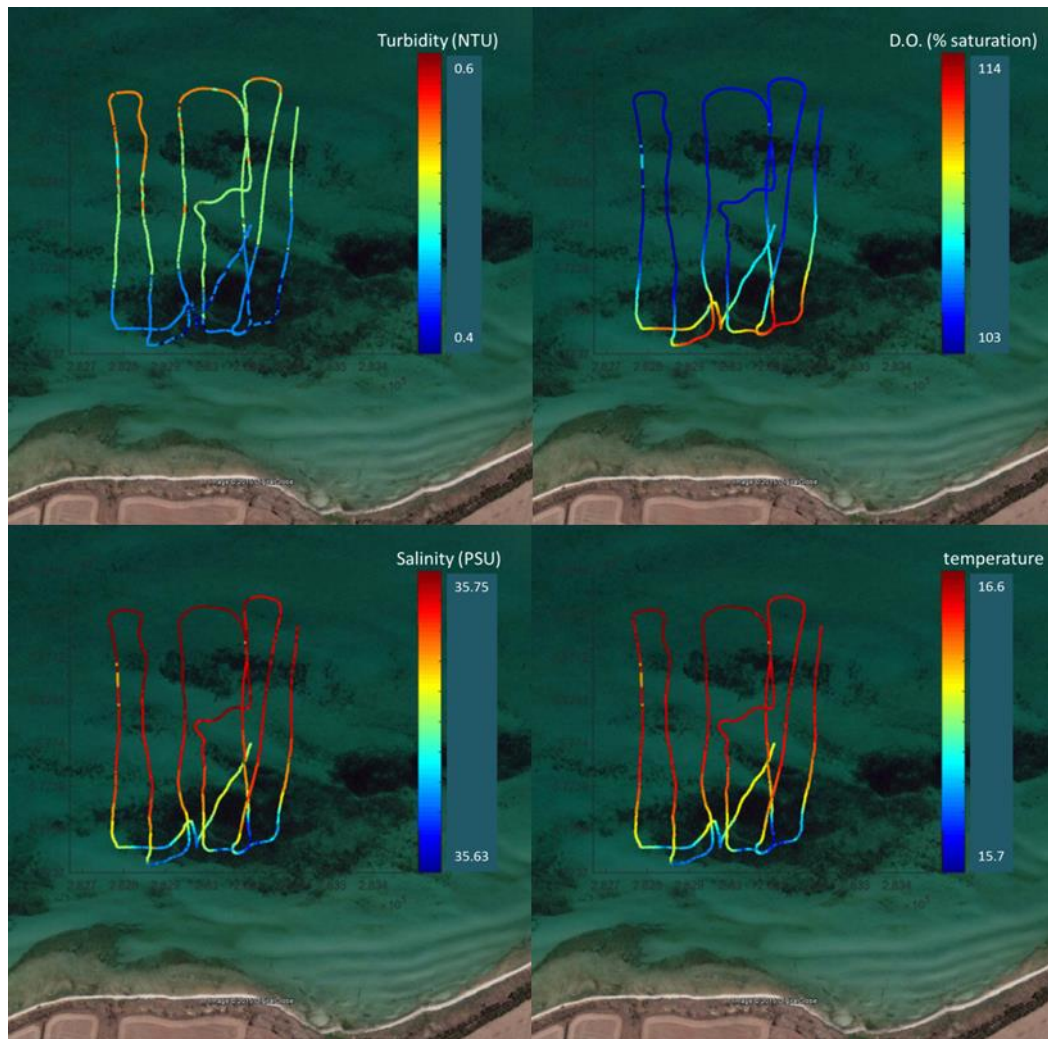
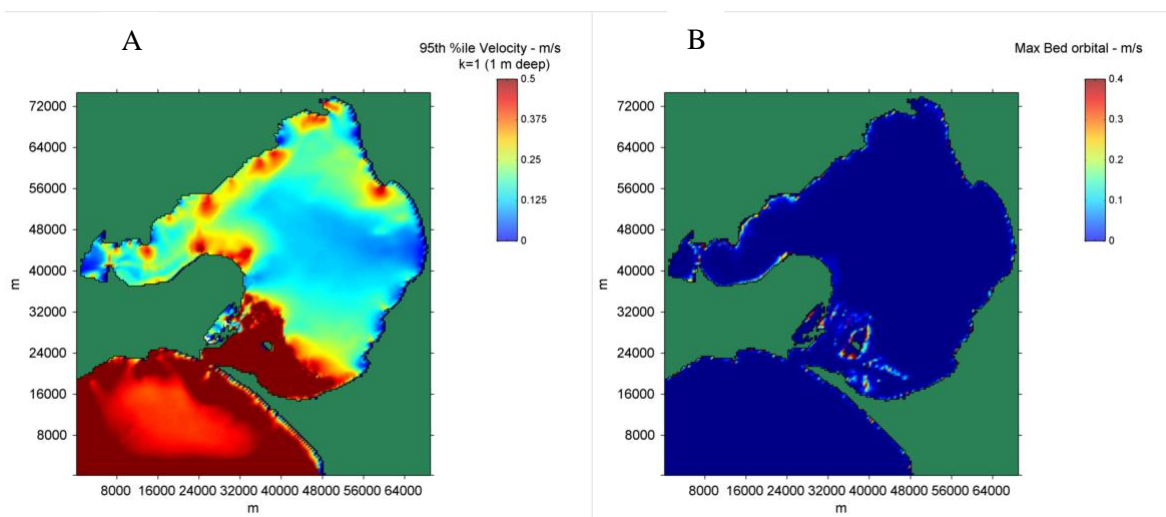


Figure 23 Spatial variation in water quality parameters at the large seagrass patch at Clifton Springs 0.3m below the water surface.

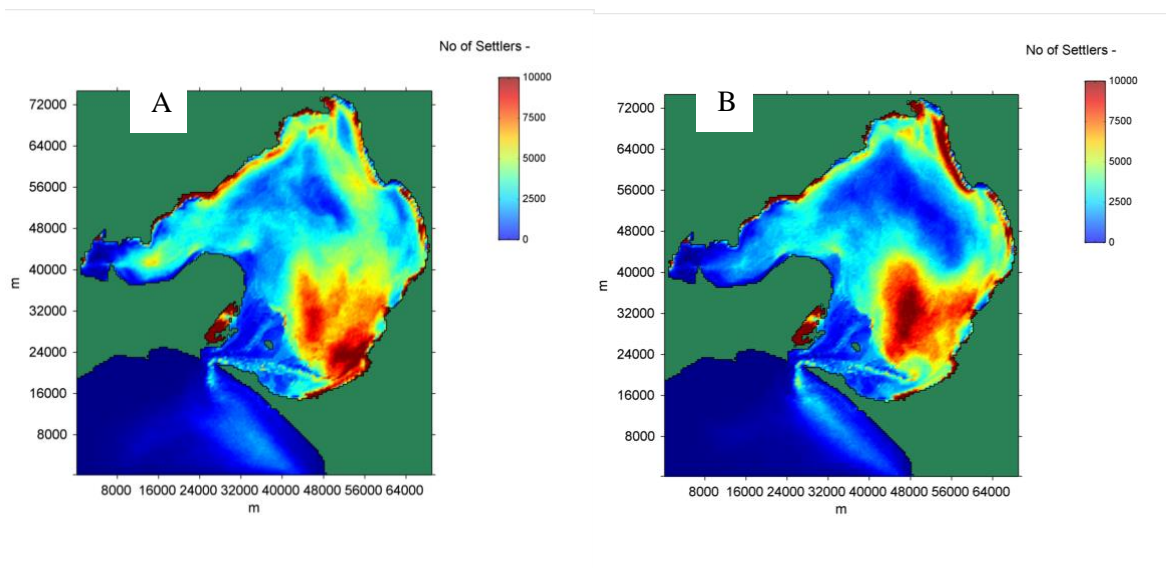
## Sediment movement

Sediments from the catchment or within the bay may be suspended by wave action and moved by currents, potentially smothering seagrass and changing seagrass distribution. The bay-wide hydrodynamic model showed that currents in the bay are strongest in the southern area inside the Heads, and drop off rapidly over the Great Sands area (Figure 24). North of the Sands the strongest currents occur near coastal features such as headlands. The orbital velocities (Figure 24) are a measure of the strength of wave action in lifting sediment off the bottom. Areas such as the Werribee coast and the Bellarine Bank have a combination of shallow water and wind fetch that results in strong wave effects on the bottom sediments.

We then used the model to simulate where sediments settle in the bay in two time periods (Figure 25). The effect of the more prevalent SW winds during the 2010-2011 period is reflected in the settled sediment results, with greater concentrations along the east coast of the bay. In both years the large pool of settled material is associated with the main current gyre in the south-east of the bay (Figure 25).



**Figure 24 Model simulations showing A) 95th percentile surface velocity and B) maximum bed velocity for July 2010-2011**

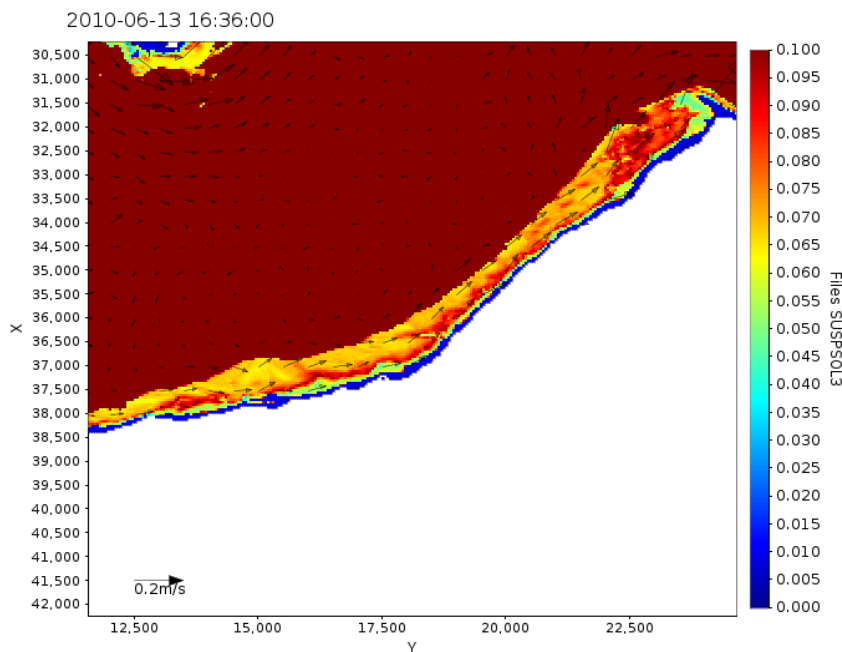


**Figure 25 Settled sediment for the representative A) dry (1999-2000) and B) wet (2010-2011) years.**

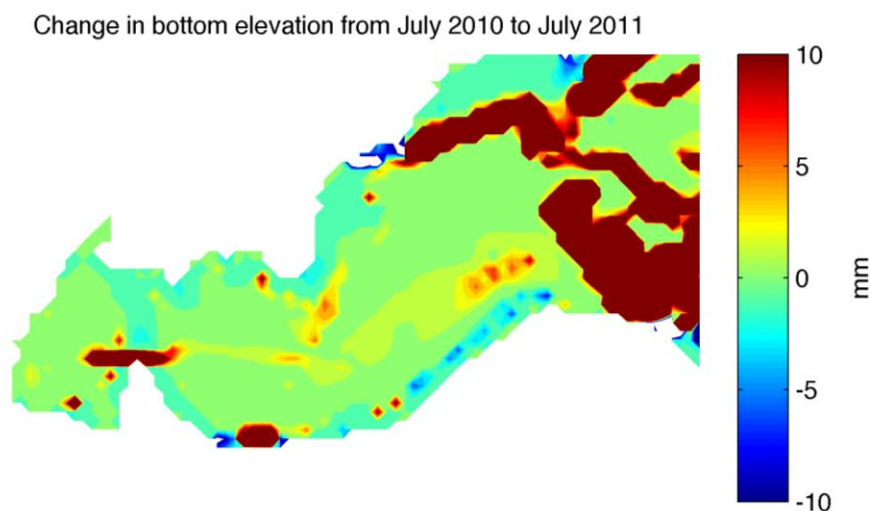
### Sediment movement and seagrass coverage along the Bellarine Bank

The Bellarine Bank is an area characterised by dynamic sediment movement and also highly variable seagrass growth and coverage, and as such was considered an ideal site to study effects of sediment movement on seagrass. Model results focussing on the Bellarine Bank during 2010 suggest an eastward flux of fine sand (Figure 26). The model estimates an eastward flow of material of  $\sim 0.1 \text{ m.s}^{-1}$  and concentrations of suspended fine sand near the seabed of  $\sim 0.075 \text{ g.m}^{-3}$ . For the  $\sim 500 \text{ m}$  wide bank would mean approximately 650kg of sand would move through the area over a day.

The model simulations for the net sediment movement of fine sand are also shown as bed elevation loss or gain in Figure 27, indicating that there are dominant erosional zones along the Bellarine Bank with 1-6mm in bed loss in 12 months (July 2010-June 2011). Time series analyses indicate that losses are continual at Pt Richards (tidally dominated), whereas for Clifton and Pt Henry they become more episodic (wind-wave driven).

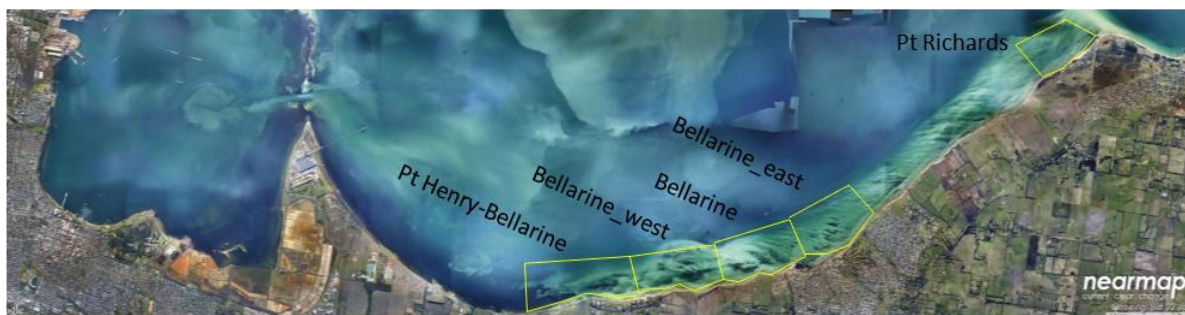


**Figure 26** Regional sediment transport modelling on the Bellarine Bank, arrows show velocity of sand movement while colours indicate sand concentration.



**Figure 27** Change in bottom (seabed) elevation due to fine sand deposition (red) and erosion (blue) zones are shown for July 2010 to June 2011.

We also used aerial photographs (nearmap) to study the relationship between sediment movement and seagrass coverage on the Bellarine Bank (Figure 27). The seabed in this area is characterised by sand bars that run approximately 45 degrees to the coast (Figure 28), with very near-shore bars running parallel to the coast in some areas (Figure 28).



**Figure 28 locations of aerial photograph analysis of sediment movement and seagrass cover on the Bellarine Bank**

The bar ridges most likely form in storms as movement of large volumes of sand would be required to form these features. Seagrass along the Bellarine Banks at present is characterised by discrete meadows that colonise the swales between the bar ridges (Figure 28). Image analysis indicated that the bars migrate along the coastline with prevailing currents towards the northeast at approximately  $4 - 5\text{ m y}^{-1}$ , causing loss of seagrass due to smothering by advancing sand ridges. Colonisation of new swale area occurs on the downstream edge of meadows, most likely via rhizome extension, allowing the meadow to migrate at roughly the same rate as the swale structures. The nearshore parallel bars can also have seagrass colonisation of the swales when conditions are suitable. Strong erosion of sand on the bar ridges most likely excludes seagrass growth. In the Point Richards area there was evidence of significant seagrass burial from sediment deposition.

Seagrass patches can increase or decrease in density and size in short time periods independently of any bar movement (Figure 29). Some of this change is seasonal with growth occurring in the spring/summer and dieback in the autumn/winter, but other changes are not seasonal and likely depend on the prevailing environmental conditions. The changes in density may occur either due to rhizome extension or re-shooting of above ground biomass from dormant rhizomes. The relatively large changes in above ground biomass suggest a strategy to deal with living in a relatively nutrient poor environment whereby plants minimise the metabolic demands during periods of low light and nutrient supply. The colonisation of new areas depends on factors such as 1) the availability of suitable substrate, 2) the coincidence of optimal light and nutrient supply, and 3) viable plants to provide for rhizome extension. A conceptual model of seagrass growth in relation to sediment movement on the Bellarine Bank is shown in Figure 30.

### **Seagrass responses to burial**

Seagrasses experience regular burial and erosion events and are constantly exposed to environmental pressures from wind and waves, sand bar migration, animal burrowing and storms. The impact of regular inundation by burial events can cause significant loss and fragmentation of seagrass meadows, but the ability of seagrasses to tolerate and recover from these events is essential to their longevity. The level of resilience and tolerance to burial is often highly species-specific and burial thresholds vary significantly between species of seagrass.

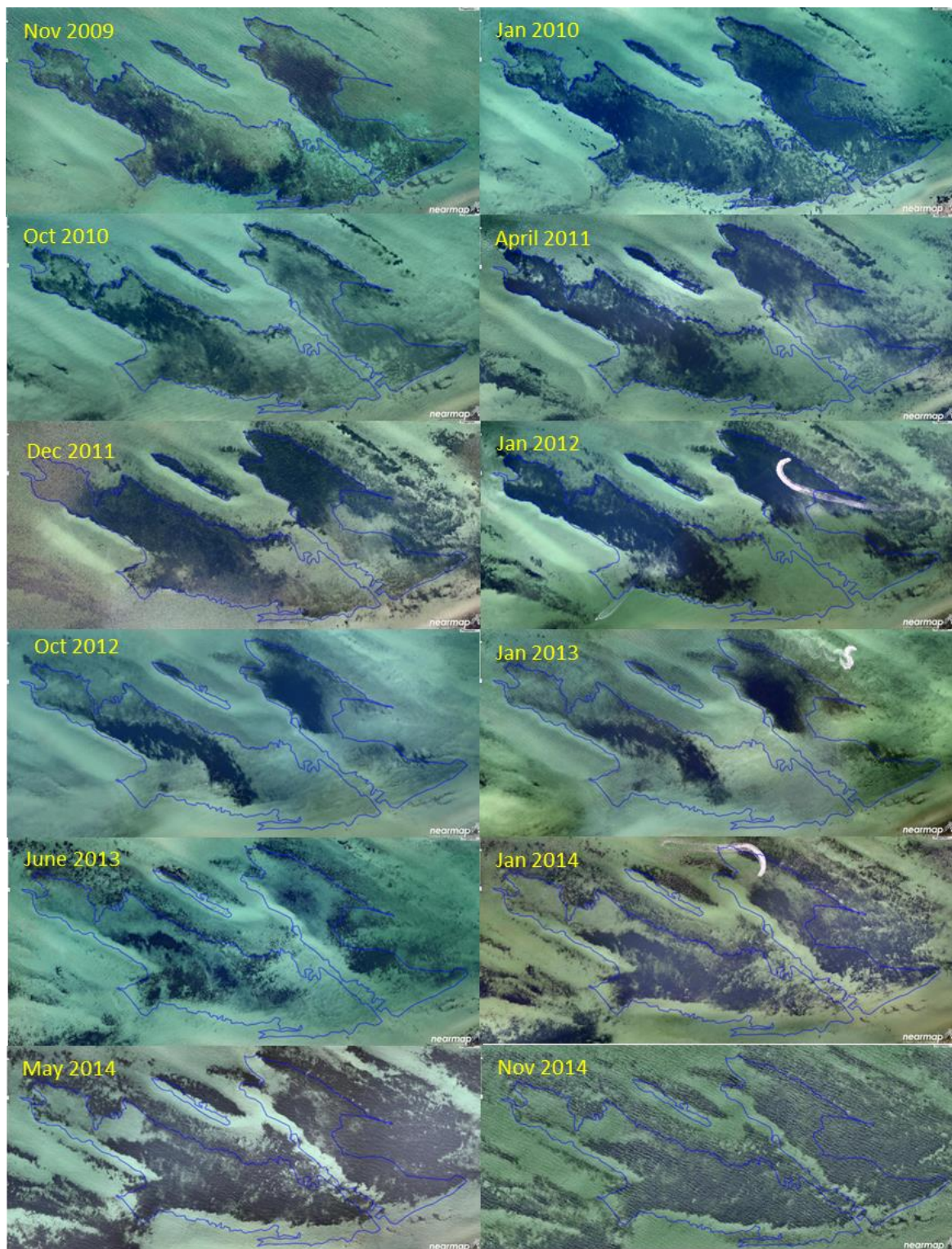
Burial thresholds for *Z. nigricaulis* in Port Phillip Bay were explored by examining the impact of burial on seagrass using experimental manipulations of varying depths and by investigating the capacity of seagrasses to recover from burial events. *Zostera nigricaulis* was able to withstand burial up to 2 cm in depth, but suffered significant mortality when buried beneath between 4 and 8 cm of sediment (Figure 31). We attributed this pattern to the presence of a modified vertical, woody stem, which allows *Z. nigricaulis* to tolerate low levels of burial. Above 4 cm burial, sediment increasingly



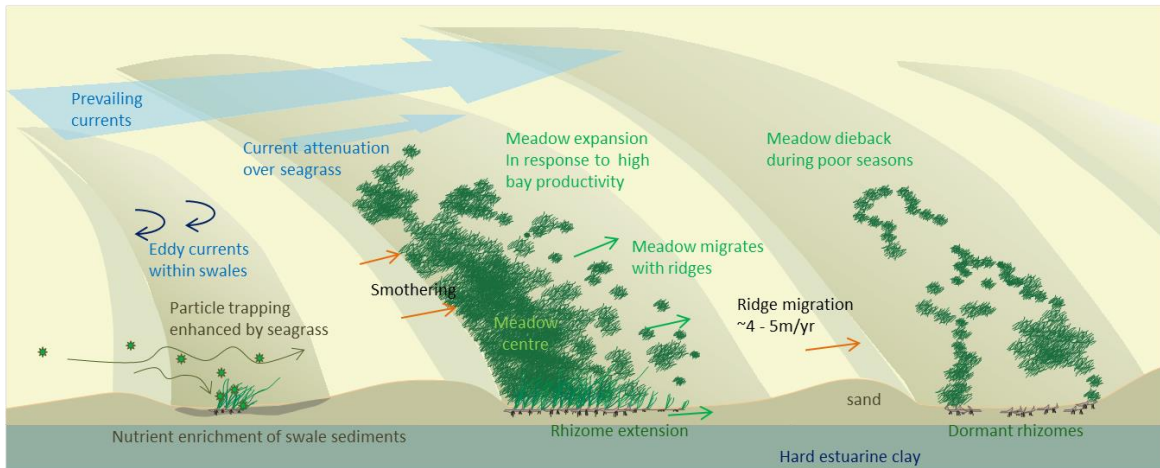
covers areas of seagrass leaf tissue resulting in higher levels of seagrass mortality relative to the experimental control. Recovery was slow, requiring 6-months for % seagrass cover to reach similar levels to that of the control or longer for seagrass length and canopy height. This implies that although *Z. nigricaulis* tolerance to burial is greater than many other similar species, its resilience following such events is poor.

The Bellarine Bank lost approximately 90% of its seagrass cover between 2000 and 2011. Prior to 2000, this region supported one of the largest expanses of seagrass in Port Phillip Bay. Analysis of satellite imagery has shown a significant change in sand bar structure along the Bellarine Bank between 2003 and 2010 reflecting a change in the wind/wave climate along this coastline. Seagrass extent along this coastline peaked in 1978, with the loss of, first offshore (1978 compared with 2000), then onshore seagrass beds (2000 compared with 2010) (Figure 32). We have presented evidence that this region is subject to nutrient limitation, and to some extent these losses are likely to be related to the millennium drought. However the dynamic nature of sediment movement in the area may also have meant that shifting climatic conditions contributed to seagrass burial and loss.

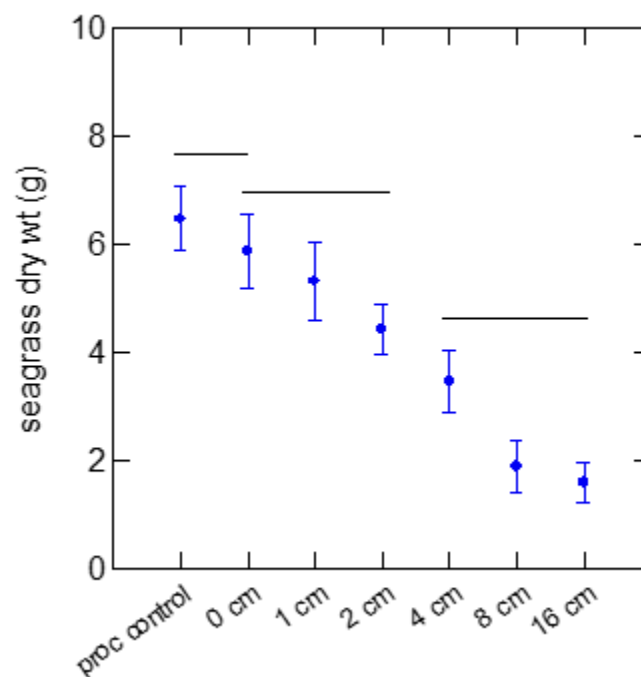
It is not clear why seagrasses have not re-colonized this region, but changes to wave regimes, dune migration and absence of the stabilizing properties of seagrass meadows may preclude significant re-colonization along this coastline. Historical analysis of seagrass trends on the Bellarine Bank indicate it took 16 years after 1946 to reach 45% of the seagrass cover recorded in 1978, but only a further 4 years to reach 90% of the cover recorded in 1978 (Figure 33). Our fine-scale hydrodynamic modelling of the effects of different size seagrass patches on currents and waves that shows that a minimum seagrass patch size of 200 m diameter is required to reduce modelled wave height along this coastline, one of the key physical constraints for seagrass distribution in Port Phillip Bay. Hence, re-colonisation of this subtidal bank may be substantially impeded by the time required for a sufficient number of seagrass patches to establish, grow and reach this size.



**Figure 29** Selected images from the Bellarine Banks showing seasonal and inter-annual variation in seagrass coverage. The blue polygons show the boundary of meadows at the start of the time sequence.



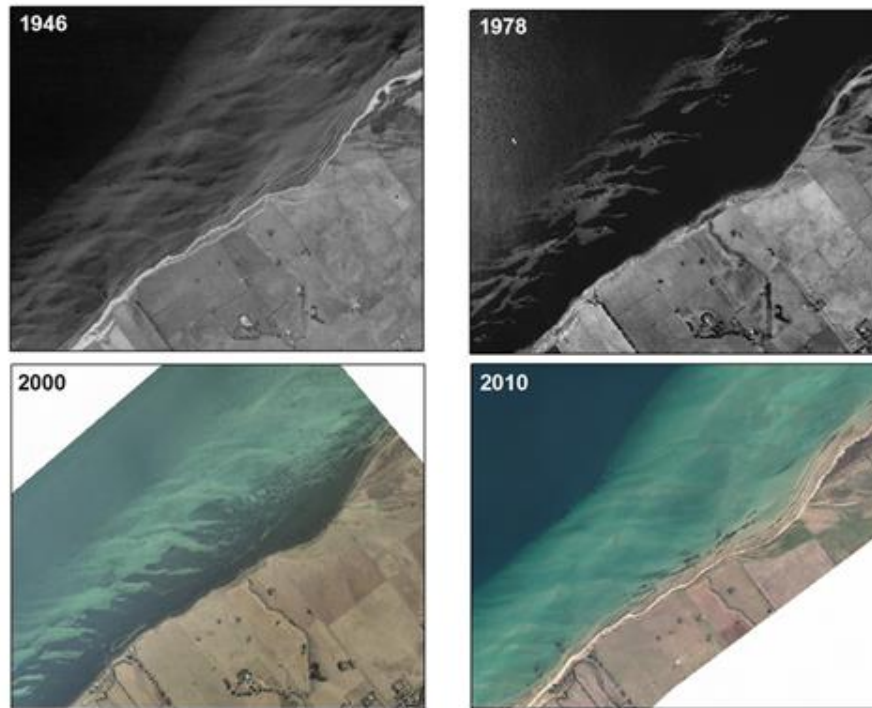
**Figure 30 Conceptual model of seagrass growth in relation to sand bar movement on the Bellarine Bank**



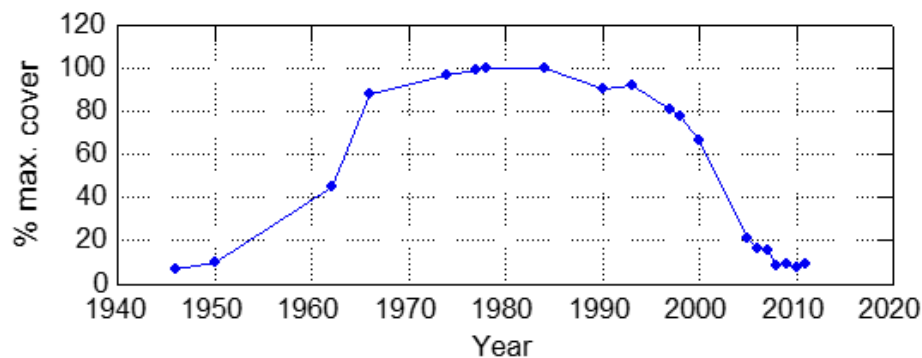
**Figure 31 Mean ( $\pm$  se) seagrass dry wt (g) following burial at 14 days at 0, 1, 2, 4, 8 and 16 cm levels and for the procedural control. Horizontal bars indicate treatments not statistically significant at  $P<0.05$  (Tukeys HSD post-hoc comparisons).**

Vulnerability to burial is unlikely to be uniform across Port Phillip Bay. First, the capacity to withstand burial may vary with seagrass canopy height and above ground biomass as it does between species. This study found that 50% seagrass mortality occurred following burial at 33% of the canopy height (Figure 34), indicating that response to burial may be dependent on the height of seagrass canopies at different locations.





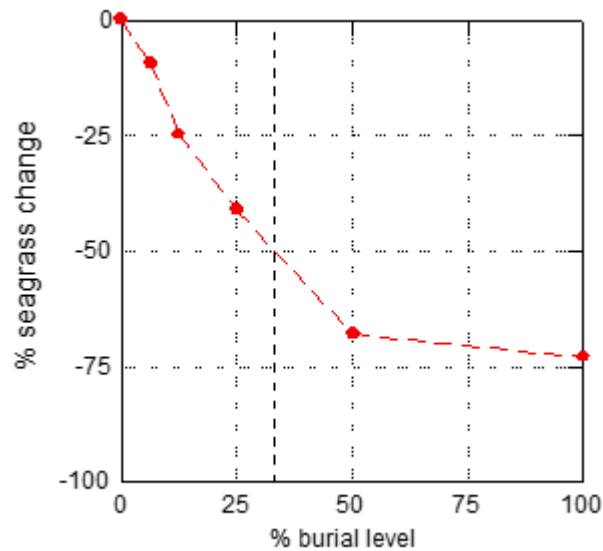
**Figure 32** Historical sequence of aerial photography taken along a section of coastline on the Bellarine Bank in 1946, 1978, 2000 and 2010.



**Figure 33** Historical trend in % maximum seagrass cover for a section of the Bellarine Bank using aerial photography from 1946–2011.

Second, seagrasses occupy regions that are prone to varying degrees of erosion and sedimentation. We used sediment transport modelling to identify areas of high sediment deposition and erosion. We suggest that shorter seagrass canopies in areas prone to high net sediment deposition are more vulnerable to burial than seagrasses with taller canopies in areas with low net sediment flux such as Swan Bay or Point Henry. Historically, the latter, have contained the most stable areas of seagrass area in Port Phillip Bay. In contrast, seagrasses growing along the open, more exposed coastlines of the central and southern margins of Port Phillip Bay are subject to higher exposure from winds/waves, currents and sediment transport. This, together with nutrient limitation, may largely explain why seagrasses in regions such as Blairgowrie, St Leonards and the Bellarine Bank have shown much higher historical variation in seagrass area than other parts of Port Phillip Bay.





**Figure 34 Relationship between % burial height and % seagrass change showing burial level at which 50% mortality is expected to occur (horizontal line).**

### 3 How do seagrasses recover?

#### 3.1 Recovery from disturbance

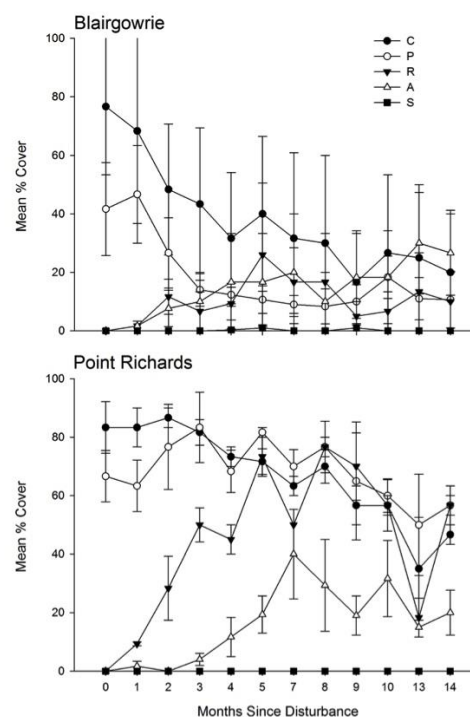
Recovery from disturbance is a key element of resilience. Local conditions influence recovery mechanisms, speed and subsequent ecosystem function. With many ecosystems, there is also a concern about the existence of alternative ecosystem states, where disturbance does not result in a return to a pre-existing condition, but instead a shift into a new condition, which itself may be persistent. In some of the best-known cases, this alternative state is less desirable, and in the case of coastal seagrass habitats, the alternative state is one with bare sediment.

We created small-scale disturbances of various kinds to look for a “tipping point” into permanently bare seabed and to determine the rate of recovery and identify the main mechanisms that underpin recovery. In particular, we created disturbances where recovery could only occur from colonists arriving (via seeds or fragments) and others where recovery could come from vegetative extension of neighbouring plants. We repeated these experiments in seagrass meadows at 10 sites in 5 regions across PPB. Recovery time varied between individual sites, but we saw no consistent regional patterns. Recovery to control levels of seagrass cover from low intensity disturbance (removal of above ground biomass) took between 2 months at Altona, Williamstown, Blairgowrie and Swan Bay North and 8 months at Swan Bay South while high intensity disturbance (removal of above and below ground biomass) took between 2 months at Altona and Blairgowrie and 13 months at Point Richards and Swan Bay South (Figure 36). The lack of regional patterns in recovery illustrates the impacts that local conditions have on growth rates and recovery of seagrass at small scales.

Recovery could occur in a couple of ways. We defined recovery as the point when experimentally disturbed areas and controls converged, and could happen when disturbed areas returned to full cover (e.g. Point Richards and Point Henry) or if changing seagrass condition throughout the experiment resulted in decreasing cover (e.g., Kirk Point, Avalon and Swan Bay North). Long dense seagrass at Point Richards and Point Henry may have hindered recovery by preventing light reaching experimental plots.



**Figure 35** Sites and regions within PPB where samples were collected to assess seagrass reproduction and morphology. Regions are North (dark blue), South (red), Central (orange) Swan Bay (yellow), Geelong Arm (purple) and Outer Geelong Arm (light blue)



**Figure 36** Seagrass recovery following several kinds of disturbance at two contrasting sites. The figure shows average seagrass cover in five different treatments, ranging from undisturbed areas (C) to areas where seagrass was removed and rhizome extension prevented (S).

Regardless of how fast recovery occurred, the recovery mechanism was similar. There was no evidence of recovery from sexual reproduction at any sites, regardless of the presence of seeds in the sediment or flower production. Prolific flowering, high densities of seeds in the sediment, and high genotypic diversity at sites such as Point Henry, Avalon, Point Richards and Blairgowrie suggest that

seeds are an important component of seagrass persistence, but the lack of recovery from seeds in disturbance experiments raises interesting questions of the role that sexual reproduction plays in seagrass recovery. Small disturbances may not facilitate recovery from seeds as recovery is inhibited by reduced light, smothering by drift algae and seagrass or excessive burial that may be avoided at larger scales allowing seeds to germinate and seedlings to grow. Although no recovery from seeds was recorded it is difficult to dismiss their importance considering the high levels of reproductive effort and genotypic diversity at some sites.

Seagrasses are highly effective at storing carbon and disturbance and recovery of seagrass could affect carbon storage capabilities. Two years after experimental disturbances, we measured sediment organic carbon was sampled at a high (Avalon) and low (Point Richards) organic carbon site. There was no difference in sediment organic carbon stocks between our different kinds of disturbance. However, losses of organic carbon from larger scale disturbances might be more important and more readily detected. Whole-meadow losses would be expected to have higher organic carbon losses due to reduced sediment stability, which would expose organic carbon within sediments to hydrodynamic forces that could cause scouring and erosion. Disturbed areas in this study are likely to be buffered from sediment (and thus carbon) loss to a large extent by the surrounding meadow. As disturbance size increases, we would expect to reach a threshold at which organic carbon losses would occur.

### 3.2 Reproduction

We measured when seagrasses flower and produce seeds around the Bay. Flower (spathe) production, timing and development, seed bank density and persistence, seed germination and morphology, and seagrass structure were all investigated at multiple sites to determine if there are variations in levels of reproductive effort and success across PPB. Initially, we used the same 10 sites as for disturbance experiments, plus two additional sites in the southern section of the Bay and two within the Jawbone and Ricketts Point Marine Sanctuaries. (Figure 35).

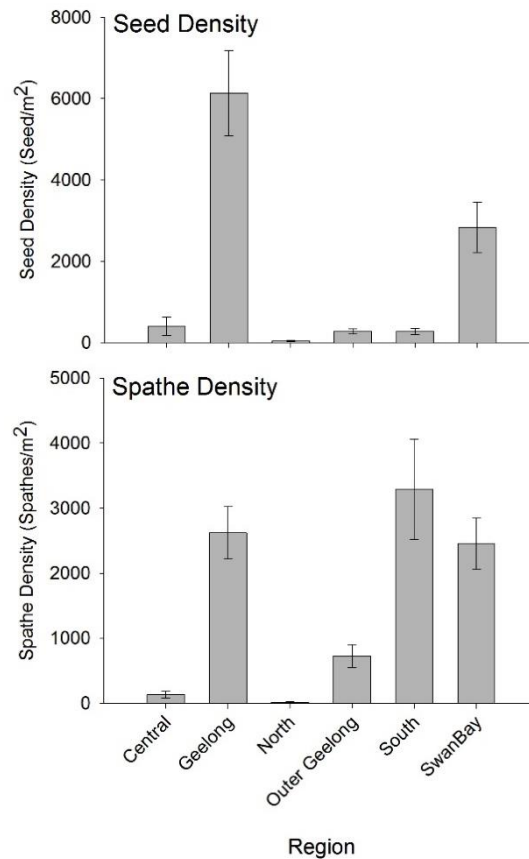
Overall, seagrass flowering and seed production occurs in late spring with a peak in flowering in September to October each year. There are big, consistent differences around the bay, with seagrasses at some sites flowering regularly and prolifically, while those at other sites flower rarely. Sites with a strong history of flowering often have large numbers of seeds buried in sediments.



**Figure 37** Seeds of *Z. nigricaulis* prior to release

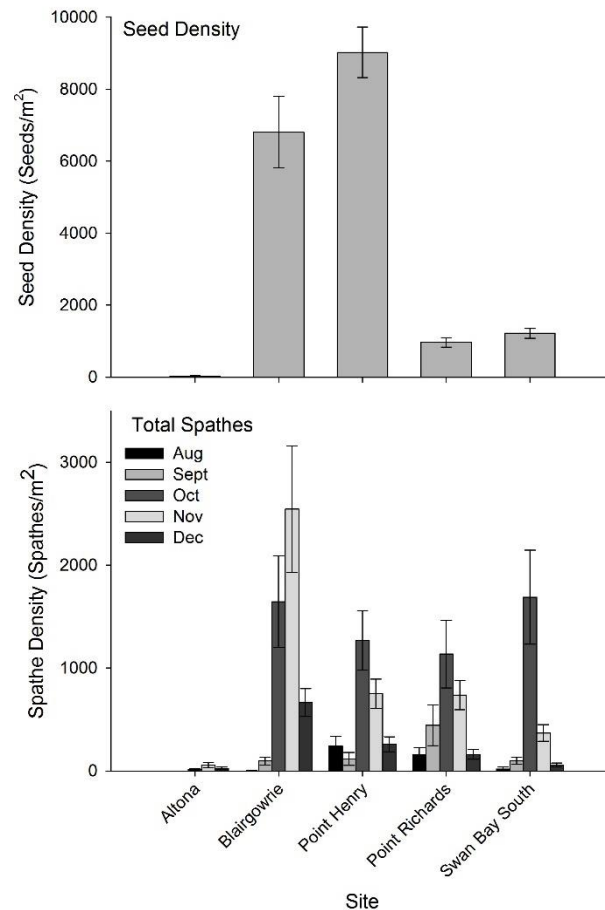
The number of flowers was very low at the two Marine Sanctuaries, Jawbone ( $7 \text{ spathe/m}^2 \pm 5 \text{ SE}$ ), Ricketts Point ( $14 \pm 7$ ), Altona ( $30 \pm 18$ ) and Mud Islands ( $30 \pm 19$ ), while Blairgowrie ( $3406 \pm 1023$ ) and Avalon ( $3220 \pm 716$ ) had the highest densities. Spathe density was significantly higher in the Geelong Arm, Swan Bay and the South than the North and Central regions of the Bay and the Outer Geelong Arm was greater than the North (Figure 38). Seed numbers showed similar patterns, being very low at Jawbone ( $27 /\text{m}^2 \pm 18 \text{ SE}$ ), Altona ( $42 \pm 29$ ) and Ricketts Point ( $56 \pm 37$ ) in the North as well as Edwards Point ( $14 \pm 14$ ). The highest seed density was recorded at Point Henry ( $10819 \pm 1774$ ) and Avalon ( $4389 \pm 1188$ ) and seed densities were significantly higher in the Geelong Arm than North and Central regions of PPB (Figure 38). The low spathe and seed densities in the

North and Central region indicate that these areas may not have the ability to colonise new areas or use seeds as a recovery mechanism in the event of disturbance. In contrast, seagrass in the Geelong Arm and Swan Bay invest heavily in the seed bank. Although the seed bank was low in the South, high spathe densities suggest that seagrass in the South invest heavily in sexual reproduction when conditions allow, replenishing the seed bank.



**Figure 38. Average ( $\pm$  SE) seed and spathe density at each region across PPB.**

Further sampling was undertaken at 5 sites (Altona, Blairgowrie, Point Henry, Point Richards, Swan Bay South) over 3 years to determine if patterns observed in the initial survey were consistent over time. Flowering was consistently rare at Altona and flowers only present between October and December over the 3-year period sampled. Across all other sites flowering occurred from August to December, peaking in October, with the exception of Blairgowrie where density peaked in November and was higher than at any other site or month (Figure 39). The seed bank persisted throughout each year but density at Altona continued to be very low ( $24 \pm 13$ ) while Blairgowrie ( $6803 \pm 995$ ) and Point Henry ( $9019 \pm 700$ ) had consistently high seed densities (Figure 39). Consistently high seed and spathe densities at Point Henry in the Geelong Arm and low densities at Altona in the North reinforce findings from the initial survey that the seagrass in the Geelong Arm is able to reproduce sexually in vast numbers, creating a mechanism for future recovery and adaptability while seagrass in the North is unable to invest resources into sexual reproduction to develop such a mechanism. The seed bank at Blairgowrie was low initially but after continual strong flowering seasons increased to very high levels, showing that flowering and seed densities may change according to variations in environmental conditions. Densities of seeds and spathes found in this study are much higher than those recorded for *Z. nigricaulis* in Western Australia but are consistent with other species of *Zostera* sampled in Japan, North America and Northern Europe, which also showed spatial variability.



**Figure 39. Average seed density ( $\pm$  SE) sampled every 3 months from October 2011 until January 2014, and average spathe density ( $\pm$  SE) at each site during each month from August to December during the same time period.**

Seagrass structure varied across PPB but showed no consistent patterns across regions. Above ground seagrass biomass, epiphyte load, seagrass length and stem density were generally greater in summer than the rest of the year, consistent with previous studies that show growth is greatest in spring and summer. Point Henry generally had the greatest seagrass length and epiphyte biomass but the lowest stem density, while Blairgowrie generally had the highest above ground biomass and stem density and Swan Bay South the shortest seagrass and lowest biomass. Biomass, length and density were similar to those recorded previously for *Z. nigricaulis* in PPB, Western Port and Western Australia. Spatial variation in seagrass structure is common, even at small scales, and likely related to environmental variables.

The presence of seeds alone does not guarantee seagrass the establishment of new seedlings; seeds need to be viable, stimulated to germinate, and become established. One experiment looked at germination rates in relation to exposure to a freshwater pulse followed by different salinity and temperature treatments. A second experiment compared different sediment types and burial depths. Overall germination rates ( $< 10\%$ ) were considerably lower than for many other *Zostera* species. Seeds that were given a low salinity pulse for 24 hours at the beginning of the experiment germinated much faster than those that were not. Germination was greater in lower salinity samples (25, 30 PSU) and temperatures ( $14^\circ$ ,  $17^\circ$ ) than in higher salinities (35 PSU) and temperatures ( $20^\circ$ ). These results suggest seed germination occurs in winter when water temperature and salinities are low and space becomes available in meadows as seagrass biomass decreases. Sediment type did not affect germination success, but seeds on the sediment surface germinated more quickly than those buried in 2 cm of sediment, further reducing the proportion of seeds that might successfully grow into adults.

This work was added to the original scope of the project, so the results should be regarded as preliminary, because it is possible that other environmental cues may produce higher germination rates (or that overall germination rates are generally low).

### 3.3 Dispersal

Seagrasses can reproduce both sexually through the production of seeds and asexually through vegetative propagation. Sexual reproduction benefits individuals by genetically diverse propagules that have an advantage in changing conditions. This diversity can also make populations more resilient to disturbance. Vegetative propagation on the other hand allows clones to persist and spread, and may be an advantage in relatively consistent conditions. Vegetative propagation also allows quick recovery from small-scale disturbances. Both reproductive modes can contribute.

Dispersal of marine organisms is notoriously difficult to observe, so we generally use indirect measures of dispersal. In this study we used genetic techniques to determine the extent of clonal (asexual) propagation and to look for links around the bay. We genotyped 720 samples from 16 sites across PPB, using microsatellite genetic markers that we developed.

Overall genotypic diversity (the number of different genotypes at a site, as a proportion of the number of seagrass samples) was 0.66, and ranged from 0.18 at Point Lonsdale near the entrance of PPB to 0.91 at Avalon within the Geelong arm. Although there was no statistically significant difference across regions in Port Phillip Bay, sites in the Geelong arm (Avalon, Grand Scenic, Point Henry, Point Richards) tended to have higher levels of genotypic diversity than the rest of the bay.

Genotypic diversity in PPB was much greater than in relatively recently colonised locations in Victoria (Apollo Bay 0.11, Portland 0.09) but similar to other bays and inlets in Victoria (Andersons Inlet 0.84, Western Port 0.79) and Tasmania (Hobart 0.90, Triabuna 0.42). Genotypic diversity was positively related to both seed bank density and spathe density in PPB, demonstrating high reproductive effort increases genotypic diversity.

Although individual seagrass areas are diverse, we found evidence for limited connections between seagrass sites around the Bay. A commonly used genetic statistic produced values suggesting limited exchange of genetic material ( $F_{st} = 0.061$ ), and most comparisons between pairs of sites suggested little exchange. The exceptions were in the Geelong Arm, where connectivity was high and the site at Rosebud could not be distinguished from most other sites. At the other extreme, Point Lonsdale, Edwards Point and North Swan Bay, were genetically isolated and were significantly different from all other sites. Network analysis showed similar results where sites in the Geelong Arm had the highest number of connections and Point Lonsdale was isolated from the other sites remaining outside the network and Ricketts Point and Edwards Point had only 1 and 2 connections respectively (Figure 40). Another form of genetic analysis, which uses a measure of the genetic similarity between populations (Neighbour joining analysis using Nei's genetic distance) showed sites forming regional clusters and with sites in the North and Geelong arm closely related, and Swan Bay formed another distinct group. In contrast, Edwards Point and Point Lonsdale were quite isolated from the other sites (Figure 41). The northern group of sites were also distinct, within even a difference between the samples from Jawbone Marine Sanctuary and nearby sites in Altona and Williamstown.

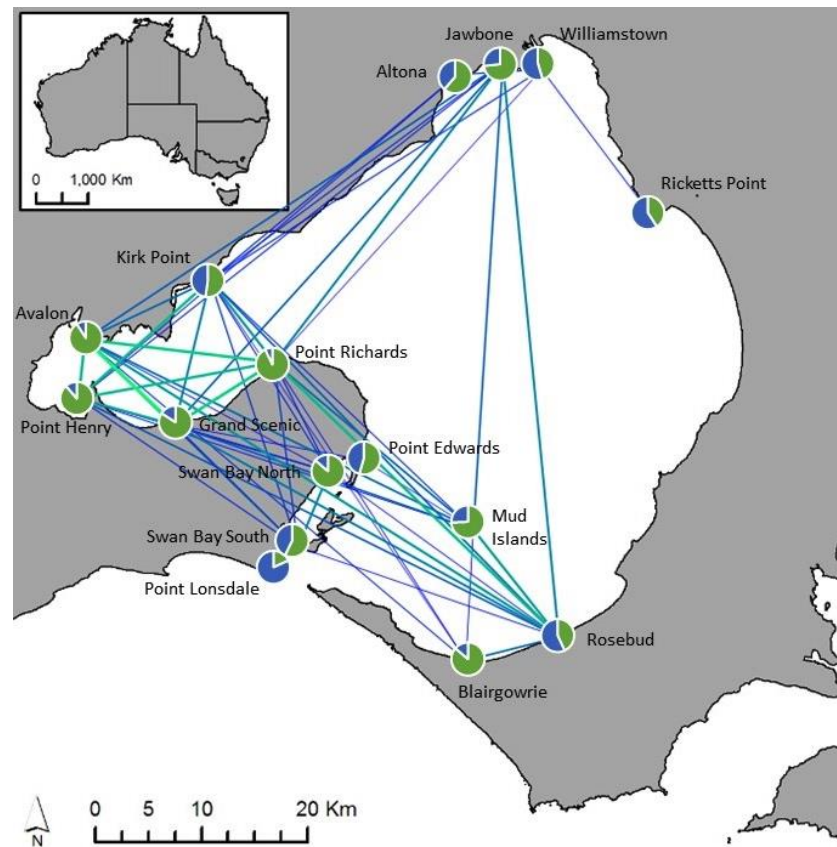
Overall, the genetic analyses show a picture of high connectivity in the Geelong Arm, where seagrasses are diverse. This area could be acting as a source area for seagrass in the rest of the bay. Some sites, however, such as Point Lonsdale, Edwards Point and Ricketts Point, are very isolated, have low genotypic diversity and widespread clones.

Although sites in different regions of the Bay were distinct, we did see signs of some broader dispersal. Matching genotypes were found at Mud Islands and Rosebud (3 different matching genotypes) and Jawbone and Williamstown, providing evidence that asexual reproduction is an important dispersal mechanism for *Z. nigricaulis*.

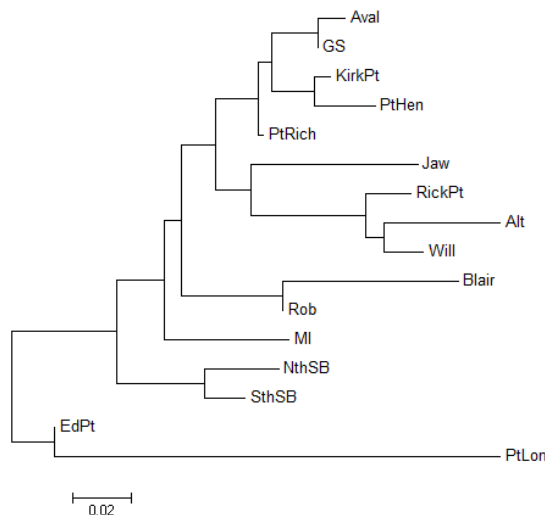
During the project, we discovered specialised asexual propagules that form on *Z. nigricaulis* branches (Figure 42) and are able to maintain buoyancy and survive in the water column for more than 3



months. While drifting, these fragments can keep growing. They do not establish very easily, but at particular times, such as following stormy weather, we found extremely high densities of propagules at sites such as Blairgowrie, Avalon and Point Richards. Our genetic data suggests that these asexual propagules do disperse and establish some times.



**Figure 40 Genotypic diversity and network analysis in Port Phillip Bay. Genotypic diversity at each site represented as proportion of unique (green) and clonal (blue) genotypes. The width and colour of the lines represents the number of connections between sites (thickness and colour from blue to green as connections increase).**



**Figure 41 Neighbour joining analysis, showing genetic similarity between *Zostera nigricaulis* sampling sites within Port Phillip Bay using Nei's genetic distance.**



**Figure 42 Asexual propagule of *Z. nigricaulis***

## 4 Key Findings

### 4.1 One bay or several regions?

#### Evidence

Our work has produced a number of lines of evidence to answer the question of whether seagrass in Port Phillip Bay is largely homogeneous or whether it has different characteristics in different parts of the bay and whether there are recognizable ecological units.

Our studies on the influence of nutrients and sediments using modelling, chemical (stable isotope) analyses and experiments have shown that there are three broad categories of *Zostera* habitat within the bay. Areas of *Zostera* that are protected from current and wave exposure, and relatively isolated from the catchment, bay such as Swan Bay and Corio Bay, have relatively stable cover of seagrass



over time ('persistent' seagrass beds). These seagrasses live in fine, muddy sediments, and most of their nutrients come from internal breakdown and recycling of detritus. In contrast, seagrasses living in more exposed parts of the bay, particularly the Bellarine Bank and the southern areas of the bay, have shown major increases and declines since the middle of last century ('ephemeral' seagrass beds). Our field and experimental studies indicate that these seagrass beds are nutrient limited, and major losses of *Zostera* occurred in these areas during the Millennium drought when catchment inputs of nutrients were low. Our studies also show that these areas have dynamic sediment movements and these may have also changed in response to climatic shifts during the drought, affecting seagrass distribution. The third category of seagrass habitat occurs along the northwest coast of the bay where nutrients are derived from the Western Treatment Plant and are unlikely to be limiting, but the combination of fine sediments and wave exposure means that turbidity is often relatively high and limiting for seagrass growth.

The nature of seagrass populations also varies around the Bay. We found major differences in how seagrasses reproduce. In the Geelong Arm, we saw regular flowering, large amounts of seed production, and a genetic structure consistent with this pattern. The northern sites were also different, with little visible flowering and seed production and genetic structure that suggests greater reliance on clonal spread within existing areas. Swan Bay also appeared an area where flowering and seed production may be important. The surprising site was Blairgowrie, where we saw prolific flowering and seed production.

Despite the big differences between seagrasses in different areas of the Bay, they responded similarly to small disturbances. If rhizomes were undamaged, recovery was quick; if the patch was small, with neighbouring seagrass, recovery also occurred quickly, but when no rhizomes were available, recovery slowed dramatically. This recovery pattern was consistent around the Bay, but we did see dramatic differences in recovery rates. These differences did not follow any consistent regional pattern, but varied at a more local scale.

## Regions

Overall, these results mean that *Zostera* in the bay is strongly regionalised into areas of 'persistent' beds (e.g. Corio Bay, Point Henry, Swan Bay) and those with 'ephemeral beds' (Bellarine Bank and the southern Bay). The north-west coast of the bay may also be considered a separate seagrass region because it has different limiting factors (likely to be light rather than nutrients) compared to the other regions. These different categories of *Zostera* habitat have differing levels of resilience to changes in water quality parameters such as nutrients and sediments. 'Persistent' beds are largely independent of changes to catchment and other inputs, and sediment transport processes, and as such are relatively resilient. In contrast 'ephemeral' beds are quite sensitive to changes in catchment inputs and sediment transport processes and will be expected to continue to show major variability over time in relation to climate and other factors.

## Connectivity

Connectivity can be viewed in two important ways. One key aspect is the connections that bring nutrients (and other material) into local seagrasses. One important finding relates to nutrient uptake by 'ephemeral' seagrass beds. Here, nutrients from the catchment and other sources may not be directly used by the plants, but rather the nutrients are initially taken up by phytoplankton. The phytoplankton are then transported by currents and "trapped" by seagrass beds. The trapped phytoplankton detritus is then broken down by bacteria and nutrients are released for uptake by seagrass. The position of seagrass beds in relation to the generation and transport of phytoplankton after rainfall and nutrient input may determine when and how many nutrients they receive.

When considering 'persistent' seagrass beds, some regions are very isolated from catchment and other sources of nutrients and sediments. The broad-scale modelling shows that water movement in some parts of the bay is extremely limited. Indeed for Corio Bay, the flushing time (where all the water in the bay is replaced by new water), is in the order of 6 months (Figure 43).

The other key aspect of connectivity is the seagrasses themselves, and how dispersal occurs. This dispersal determines the capacity of particular sites to recover from substantial disturbance, particularly a disturbance that overwhelms a local seagrass meadow. This dispersal also influences local genetic diversity, which is thought to affect local populations' abilities to deal with future challenges. Our genetic analyses suggest that there are restrictions on dispersal (as might be expected with seeds that sink). Some parts of the Bay show genetic patterns that suggest regular dispersal, leading to diverse, well mixed populations. This pattern is most obvious in the Geelong region. Swan Bay also seems an area with some mixing, but it is distinct from other areas of the Bay. The northern sites are genetically distinct, though there is evidence of some connections to seagrasses in the Geelong region. We identified two other kinds of sites. Some places seem disconnected from other areas. These tend to be small areas that are isolated from other seagrass areas (e.g. Rickets Point Marine Sanctuary and Pt Lonsdale). The second sites are those with evidence of some connection with other sites around the Bay. These sites were Mud Islands and Rosebud, and to a lesser extent, Blairgowrie. Because these sites are all in the southeast, the most likely explanation is that these sites do receive colonists from around the bay, from asexual fragments (or fragments carrying seeds). Fragments can survive long enough while drifting to move there.

## **4.2 What do we still need to know?**

We have suggested that the limited and patchy distribution of seagrass on the northwest coast is most likely due to light limitation from turbidity. However, we need more data to be confident in this conclusion. Although we have determined broad thresholds of light requirements of *Zostera*, we are lacking more detailed information on the seasonal differences, the effects of different exposure times as well as many other variables. We have conducted some preliminary experimental trials in the laboratory on turbidity effects on seagrass but these need refinement. Other possible factors could affect seagrass in this region include low salinity from catchment and WTP inputs, and also epiphyte growth from excess nutrients reducing light for seagrass. We need more experimental information on the potential for decreased salinity to affect seagrass growth and for excess nutrients to cause shading of seagrass through epiphyte growth.

With the modelling tools we have developed and the greatly increased knowledge base we have of the environmental requirements of *Zostera* in Port Phillip Bay we are in a position to explore the potential threats of climate change. Projections of environmental changes to Port Phillip Bay in the future from climate change modelling by the CSIRO and Bureau of Meteorology can be incorporated into our broad-scale and fine-scale models to test the effects on seagrass. This information is available on a fine (5 x 5 m grid) that can be incorporated into the spatial structure of our models. Such environmental variables would include rainfall (amount and variability), nutrient and sediment inputs, air and sea temperature, wind strength and direction, and sea level rise.

The question of how seagrasses colonize areas following complete loss is an important one. We understand how recovery occurs from minor damage, but it is less clear what happens when all above- and below-ground material is lost over a large area. We have identified two colonization pathways, seeds and fragments and have some understanding of the characteristics of each. Overseas, there is considerable interest in "assisted" recolonization, but for this to be a useful remedial tool in Victoria, additional work is necessary. There are three aspects to this work:

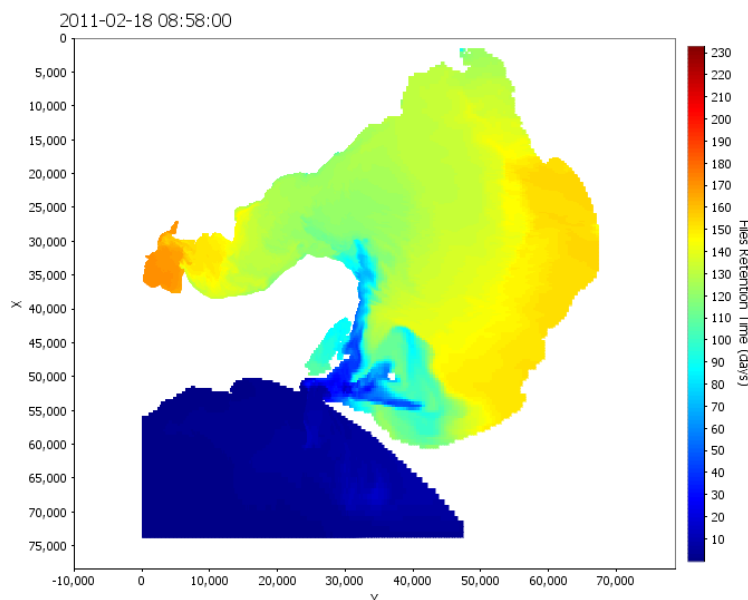
- What is the nature of seed production and germination, and are there environmental stimuli that result in high germination success? It seems paradoxical that in many areas, seagrasses produce so many seeds, but we saw little sign of germination, and we only produced <20% germination in the laboratory. Do seagrasses produce many seeds, with low germination success, because seeds are "cheap"? Are particular conditions required before germination can occur? Answers to these questions will inform managers as to whether revegetation can be enhanced with propagules, as has been effective elsewhere.
- What is the role of fragments? Again, we saw huge numbers of these fragments being produced, and it is not uncommon to see large numbers washed up after storms. We know that these fragments remain viable for a long time, but, again, in our experiments, they did not

survive well. As for seeds, are these fragments produced in large numbers because they are a “cheap” investment for an individual seagrass plant, and the payoff is the rare fragment that establishes, or are there special circumstances where establishment occurs at high rates (e.g. in particular sediment conditions, or when particular structures hold the fragment in place on the seabed)?

- We also observed drifting fragments with seeds present. These are intriguing. The fragments allow the seeds, which normally sink, to be dispersed by currents, and they have the benefit of a fragment arriving in a new area with several to many fellow (more genetically diverse) colonists. This could increase the likelihood of success. Understanding how these particular fragments disperse, and the conditions under which they establish, could help to understand the possibility of restoration.

As with any piece of scientific work, there are inevitable gaps. Some are “interesting”, while others are important. The project team made decisions about where work was to be done. Our initial decisions were made around what we knew already of the Bay and its different regions, with some consideration also of sites where we already had existing knowledge. Different project components used subsets of this large group of sites, and, while we tried to stay consistent, there were gaps. As the project developed, we became interested in some areas that were not an initial focus, e.g. the Bellarine Bank. As a result, we have a partial picture of what happens in this area. There are two spatial gaps that we have identified:

- Our focus on persistent vs ephemeral seagrass beds means that our understanding of recovery, seed banks, etc., is less complete for these ephemeral areas.
- With the high cost of doing marine field work, most of our work was done in relatively shallow, nearshore areas. This results in a gap around deeper areas, which might be an important consideration, as seagrasses in deeper water will be closer to their physiological limits. It would be interesting to know how the process of recovery from disturbance occurs in these waters.



**Figure 43 Modelled retention time for an 8 month period in 2010-2011 shows the bay has variable capacity to retain and flush.**

### 4.3 Risks & vulnerabilities

Regions with ‘ephemeral’ seagrasses tend to have to have dynamic sediment movement. The seagrass in regions such as the Geelong Arm play an important role in mitigating wave energy to the eroding coastlines such as along the Bellarine Bank. The introduction of coastal structures (breakwaters, marinas, etc) can present a significant risk to seagrass by altering the pattern of sediment transport in the area.

The flux of silts along the northwest coast of the bay and associated reductions in light clarity, indicate that this is a vulnerable location for seagrass. Future changes in outflow management of the WTP or developments of coastal infrastructure in this region should consider the sensitivity to light limitation to optimise the seagrass health in this region.

Southeastern Australia’s future climate is expected to become drier on average as a consequence of global warming. Projected decreases in rainfall coupled with increased temperature and evaporation rates are expected to reduce run-off across southeastern Australia by 20–36% by 2060. Increasing intensification of the El Nino Southern Oscillation in the western Pacific is also expected to result in higher incidence of drought in eastern Australia. It is highly likely that under such a scenario the relative importance of catchment inputs of Nitrogen for seagrass growth will diminish as N inputs from the catchment decline and become more variable over time as southeastern Australia experiences longer and more frequent periods of drought. This may have consequences for the growth, distribution and resilience of ‘ephemeral’ *Z. nigricaulis* seagrass regions in Port Phillip Bay that depend on external sources of Nitrogen.

The results of the statistical modelling imply that changing wind patterns, as may occur with shifting climatic patterns such as during drought or in the future through climate change, or changes to water clarity though changes to catchment inputs of sediments or nutrients stimulating phytoplankton growth, may lead to fluctuations in the bay-wide distribution of seagrass over time, and a permanent reduction in water clarity could lead to a permanent reduction in habitat available for seagrass. Climate change is a significant risk in this respect, as increased intensity of individual rainfall events and associated flooding may reduce water clarity.

### 4.4 Summary against performance indicators

Performance Indicator	
1. The primary sources of nitrogen utilised by <i>Zostera nigricaulis</i> in different parts of PPB are identified	Achieved, see section 2.2 (i) Nutrients
2. The nutrient status of seagrasses in PPB, particularly in relation to nutrient limitation is mapped	Achieved, see section 2.2 (i) Nutrients
3. When and how seagrasses utilise nutrients is understood through quantifying the uptake and flux of nutrients in seagrass beds	Achieved, see section 2.2 (i) Nutrients
4. The effect that elevated nutrient and turbidity levels have on seagrass and epiphyte growth (including their relative importance) will be understood, including threshold levels that lead to significant change.	Achieved*, see section 2.1 Bay-wide processes, 2.2 (i) Nutrients, (ii) Light & Turbidity  *Thresholds for nutrients not meaningful based on results, varies with site and source. Broad thresholds for turbidity determined but more work needed.
5. The relationship between large-scale hydrological	Achieved, see section 2.1 Bay-wide

(wind, waves, currents), physical (sediment type) and sediment transportation processes and seagrass distribution in PPB is understood	processes, 2.2 (iii) Sediment Movement
6. Sediment burial thresholds for <i>Zostera nigricaulis</i> seagrasses in PPB are determined	Achieved, see section 2.2 (iii) Sediment Movement
7. Region-specific assessment of seagrass beds around PPB, detailing the roles of seeds, rafting fragments, clonal growth within beds and bed size and shape in resilience of beds.	Achieved, see Section 3.2 and parts of section 2
8. An assessment of the extent to which local seagrass beds are discrete ecological units, compared to a reliance on a regional or a bay-wide network of seagrass areas. Part of this assessment was to include, if relevant, region-specific measures of seagrass recovery rates from disturbance.	Achieved, see Sections 3.1 and 3.3
9. A conceptual framework for seagrasses in PPB, incorporating regional and local water quality, sediment processes, and basic reproductive biology.	Achieved, see Section 2

## 5 Publications and Reports (Technical Appendices)

Note: Technical Appendices will continue to be updated. The most up to date version of each appendix can be found at <http://bit.ly/1M7AW12>.

1. Hirst A. J., Khageswor G, Ball D, Blake S and R Lee Determination of the physical drivers of seagrass distribution and abundance in Port Phillip Bay, Australia, using a spatial autoregressive lag model. Draft Journal Manuscript
2. Randall Lee, Dougal Greer and Alastair Hirst (2015) Developing Tools for the Management of Nutrient and Sediment interactions with seagrass ecosystems in Port Phillip Bay: Broad scale modelling, EPA Technical Report 1591
3. Nayar, S. & Bott, K. (2015). Uptake and translocation of ammonium and nitrate by temperate seagrass *Zostera nigricaulis* in Port Phillip Bay. Research Report Series No. 819, SARDI Aquatic Sciences.
4. Cook, P. L. M., Evrard, V. and Woodland R. J. (2015). Factors controlling nitrogen fixation in temperate seagrass beds. Marine Ecology Progress Series (In press)
5. Gay et al. Sediment dynamics and organic matter deposition in and around seagrass meadows along the Bellarine Bank
6. Hirst, A. J., Longmore, A. R., Ball, D., Cook, P. L. M. and Jenkins G. P. Linking nitrogen sources utilised by seagrass in a temperate marine embayment to patterns of seagrass change during drought. Draft Journal Manuscript
7. Hirst, A. J. Experimental test of N-limitation for *Zostera nigricaulis* seagrass at three sites in Port Phillip Bay, Australia, reliant upon very different sources of N. Draft Journal Manuscript
8. Ferguson, A. Temporal trends in seagrass along the Bellarine Banks
9. Randall Lee, Dougal Greer, Peter Yeates, Chris Dallimore, and Gregory Jenkins (2015) Developing Tools for the Management of Nutrient and Sediment interactions with seagrass ecosystems in Port Phillip Bay: Sediment processes and light modelling for Seagrass, EPA Technical Report 1592
10. Gay et al. Water quality dynamics in and around seagrass meadows along the Bellarine Bank.
11. Hirst, A. J., McGain, S., Lee R. and Jenkins G. P. The impact of burial on the survival and recovery of *Zostera nigricaulis* in Port Phillip Bay, Australia. Draft Journal Manuscript
12. Smith, T. et al. Recovery of seagrass from small-scale disturbance

13. Macreadie, P.I., York, P.H., Sherman, C.D.H., Keough, M.J., Ross, D.J., Ricart, A.M. *et al.* (2014). No detectable impact of small-scale disturbances on 'blue carbon' within seagrass beds. *Marine Biology*, 161, 2939-2944.
14. Smith, T. *et al.* Spatial variation in the reproductive effort of the seagrass *Zostera nigricaulis* in Port Phillip Bay, Australia
15. Smith, T. *et al.* The role of reproduction and dispersal in determining levels of genotypic diversity in a clonal organism
16. Sherman, C.D.H., Stanley, A.M., Keough, M.J., Gardner, M.G. & Macreadie, P.I. (2012). Development of twenty-three novel microsatellite markers for the seagrass, *Zostera muelleri* from Australia. *Conservation Genetics Resources*, 4, 689-693.
17. Smith, T.M., York, P.H., Stanley, A.M., Macreadie, P.I., Keough, M.J., Ross, D.J. *et al.* (2013). Microsatellite primer development for the seagrass *Zostera nigricaulis* (Zosteraceae). *Conservation Genetics Resources*, 5, 607-610.
18. Thomson, A.C.G., York, P.H., Smith, T.M., Sherman, C.D.H., Booth, D.J., Keough, M.J. *et al.* (2014). Seagrass viviparous propagules as a potential long-distance dispersal mechanism. *Estuaries and Coasts*.
19. Thomson, A. (2012). The contribution of vegetative fragments to seagrass meadow dynamics and resilience (*Zostera nigricaulis*). Honours thesis, University of Technology, Sydney
20. Cumming, E. (2013). The reproductive biology of a temperate seagrass *Zostera nigricaulis*, in Port Phillip Bay, Victoria, Australia. Honours thesis, Deakin University.
21. Gay *et al.* Seagrass biometrics along the Bellarine Bank
22. Gay *et al.* Current velocity and attenuation in and around seagrass meadows along the Bellarine Bank
23. Ferguson, A. Conceptual model for seagrass in Port Phillip Bay

## 6 Glossary

**Benthic microalgae (BMA):** Single cell plants that live in and on the sediment

**Epiphytic algae:** Benthic microalgae and multicellular algae living on seagrass plants

**Fractionation:** a change in the ratio of stable  $^{15}\text{N}$  to stable  $^{14}\text{N}$  in pore water, brought about by the preferential metabolism of  $^{14}\text{N}$  by bacteria

**Genotypic diversity:** the number of different genotypes at a site, as a proportion of the number of seagrass samples taken

**Nitrogen fixation:** the conversion of atmospheric nitrogen into nitrogen compounds by certain bacteria

**Phytoplankton:** Single cell plants that drift in the water column

**Pore water:** water in the porous parts of the sediment

**Rhizome:** a continuously growing horizontal underground stem which puts out shoots and adventitious roots at intervals.

**Seagrass:** A flowering plant evolved from a land plant that now lives permanently in marine waters

**Spathe:** the flowering structure of seagrasses

**Stable isotope:** Forms of an element with more (heavier) or less (lighter) neutrons relative to protons.

**Translocation:** Movement of nutrients between different parts of the seagrass plant (roots, rhizomes, leaves)