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Port Phillip Bay Nitrogen Monitoring Proposal- Statistical Advice

A. R. Longmore and A. Gason

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**Marine and Freshwater Resources Institute
PO Box 114
Queenscliff 3225**

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Librarian
Marine and Freshwater Resources Institute
PO Box 114
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**Phone: (03) 5258 0259
Fax: (03) 5258 0270
Email: Julie.Mather@nre.vic.gov.au**

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ABSTRACT

As part of the development of a nutrient management plan for Port Phillip Bay, a workshop was held in October 1998 to discuss possible approaches to monitoring over the next decade which may provide early warning of detrimental changes to Bay nutrient cycling. Specifically, the objective of the workshop was to propose programs:

“To provide an early warning of detrimental changes to critical elements of Bay nitrogen cycling processes that indicate an increased risk of eutrophication at Bay-wide and regional scales.”

Four programs were proposed by the workshop. The two given highest priority were for monitoring of:

1. Denitrification efficiency; and
1. Water quality

These two highest priority programs have now been further assessed for their feasibility and cost.

To a large extent, the ability of any of these programs to provide an early warning of possible changes in the nitrogen cycle will depend on the type, magnitude, duration and location of any impact. To develop relevant and targeted monitoring proposals, current scientific understanding of the indicators of impending eutrophication provided by these programs and the type and scale of change that may be ecologically important to the Bay was assessed (Longmore 2000). Based on this, appropriate and practical monitoring approaches and technologies were recommended and costed. As our knowledge of Bay nutrient cycling remains incomplete, pilot studies to address some key knowledge gaps involved in improving and interpreting these programs were also identified. Further refinement of these recommendations requires statistical analysis of existing data. In this report, that analysis was carried out, to the extent feasible from available data. The analyses (along with other scientific and practical considerations) were used to recommend appropriate site numbers and locations and sampling frequencies, given patterns and variability in the data and its implications to the level of change in these indicators that we can be confident should be statistically detectable. This provides a rigorous basis on which the proposals could proceed, with future intermittent reviews based on the improved understanding that would be derived from the incoming data.

On the basis of the available data, the number of sites recommended for benthic flux measurements by Longmore (2000) has been reduced from three to two, with at least four replicate measurements from each site needed to provide 80% confidence of detecting a significant change in fluxes. Denitrification efficiency is a more sensitive indicator of change than the other recommended indicators. Insufficient information was available to provide statistical guidance on the design of the proposed pilot studies, but suitable statistical tests were identified to apply to the resulting data.

Information from *in situ* water quality monitoring equipment is likely to be gathered at a greater rate than any phenomena we wish to understand. An appropriate statistical procedure was identified to interpret the electronic monitoring information. An assessment of existing water quality information indicated that the bay can be split into three main zones of similar water quality: northern, western and central. Three

monitoring sites are therefore proposed, which coincide with those currently sampled by EPA. Statistical analysis of existing data indicated that our ability to detect significant change in recommended indicators varies with indicator and site; while chlorophyll is the most powerful indicator of change in Hobsons Bay, dissolved organic nitrogen and particulate nitrogen are the most sensitive indicators at all three sites.

Appropriate Quality Assurance/Quality Control procedures for the proposed studies have been defined. Indicative costings have been made for the five instances where statistical analysis indicates that the monitoring proposal should be modified from that costed in Longmore (2000).

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INTRODUCTION

As part of the development of a nutrient management plan for Port Phillip Bay, a workshop of scientists with expertise in nutrient cycling was held in October 1998 to recommend key approaches to monitoring over the next decade which may provide early warning of detrimental changes to Bay nutrient cycling. The monitoring workshop identified and prioritised monitoring approaches and indicators in terms of the following objective: “*To provide an early warning of detrimental changes to critical elements of Bay nitrogen cycling processes that indicate an increased risk of eutrophication at Bay-wide and regional scales.*”

The workshop identified a number of possible approaches to address the objective. These were then assessed for feasibility (Longmore 2000), and a number of pilot studies and monitoring recommended, on the basis of past experience and our current understanding of nutrient cycling in Port Phillip Bay. Statistical refinement of the monitoring recommendations and appropriate QA/QC programs were needed before such programs could commence. In terms of statistical design, the scientific understanding about the indicators and their role in nutrient cycling provided by Longmore (2000) is needed to:

1. guide identification of an appropriate statistical model for data analysis (eg: regression model to assess trends; t-test for comparison with a criterion)
2. design a sampling program that will provide appropriate data - this includes relevant sampling scales, sites, times based on understanding of the relevant processes
3. identify levels of, or changes in, the indicators that the program and subsequent statistical analysis need to provide acceptable confidence of detecting change.

The statistical design itself, and an appropriate QA/QC program, are the subjects of this report. It focuses on the subset of monitoring approaches that were allocated highest priority at the nutrient monitoring workshop.

STATISTICAL ADVICE ON RECOMMENDED PROGRAMS

Benthic fluxes

The approach is based on the understanding that increased nitrogen inputs to the Bay will be rapidly transformed to phytoplankton biomass, a large proportion of which settles to the bottom. Bacteria on and in the sediment then begin to break down the organic matter, consuming oxygen, and returning carbon dioxide and nutrients to the water column (a nutrient flux). A close coupling of nitrifying and denitrifying bacteria within the sediment leads to the conversion of most of the recycled organic nitrogen to N_2 gas, which is lost to the atmosphere. Port Phillip Bay appears to be much more efficient at denitrification than most northern hemisphere marine embayments (Berelson *et al.* 1998), and maintenance of a high denitrification efficiency is critical to the maintenance of good water quality in Port Phillip Bay (Harris *et al.* 1996). Denitrification efficiency, oxygen and carbon dioxide fluxes were recommended as the critical indicators to monitor (Longmore 2000).

A total of 136 benthic flux measurements from 12 sites in Port Phillip Bay were available to describe patterns in benthic flux measurements and levels of background variability

against which change would need to be detected. The samples were all collected during 1994-96, as part of the Port Phillip Bay Environmental Study (Nicholson and Longmore, 1996; Berelson *et al.* 1998), and included 54 measurements from a site near the Werribee coast (Fig 1, site 6), 24 measurements from central Port Phillip Bay (site 37), and 22 measurements from Hobsons Bay (site 16). Analysis of variance (SAS General Linear Models procedure, using type III sums of squares) indicated that there were statistically significant differences between sites, and between seasons, for all fluxes. Specifically, Tukey's Studentised Range (HSD) test indicated that mean ammonium, phosphate, silicate, carbon dioxide and oxygen fluxes were higher in summer than winter, while mean denitrification efficiency was lower in summer than winter.

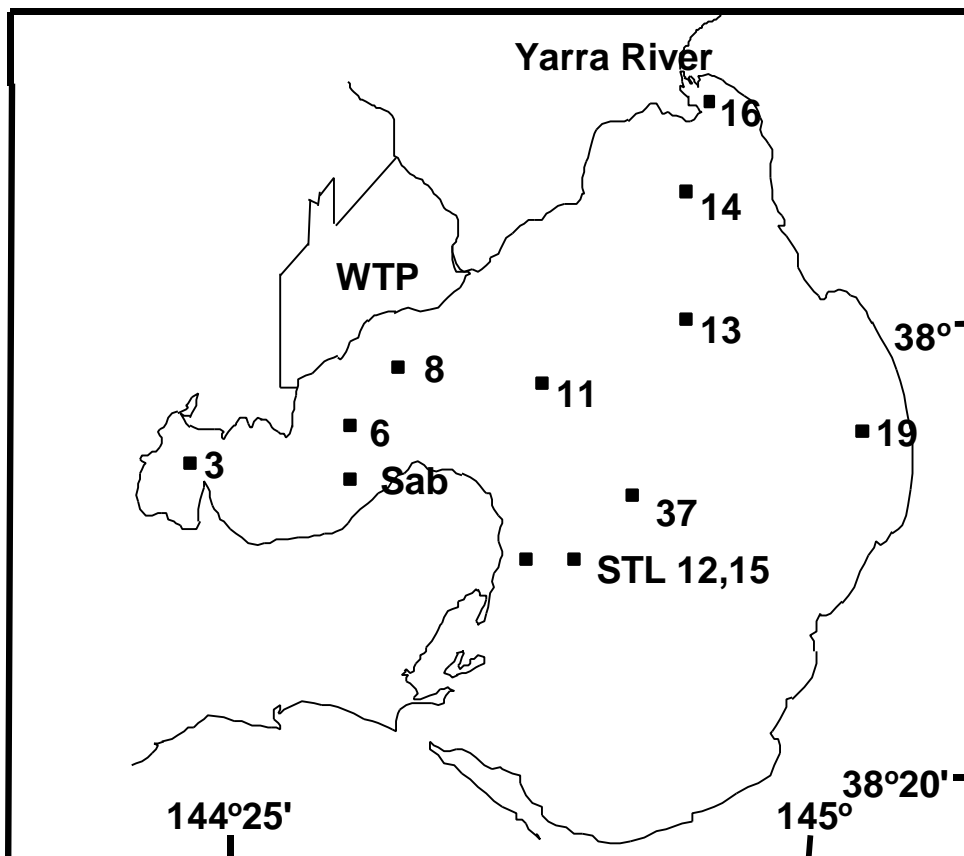


Figure 1. Benthic flux sites sampled during 1994-96.

Fluxes were tested for homogeneity of variance using Levene's test, and log-transformed if necessary. The number of samples required to detect a 10-200% change in a variable at 0.05 significance level (with 80% power) using a simple t-test comparing data from different sampling periods at each site was calculated for dissolved oxygen flux, carbon dioxide flux, ammonium flux and denitrification efficiency from sites 6, 16 and 37 (Appendix 1). Where significant seasonal variation occurs (which ANOVA indicates is the case for the four indicators listed above), t-tests comparing means for the same season from different years should be capable of detecting smaller changes than those indicated in Appendix 1. However, there is not yet sufficient data to determine if fluxes are consistently different between seasons over a number of years.

Longmore (2000) recommended using absolute denitrification efficiency values to interpret significant level of change, but it may be preferable to set percentage changes, for comparison with CSIRO model outputs. Murray and Parslow (1996, Table 8.4)

estimated that a Bay-wide change in D_{\max} (the proportion of sedimentary ammonium which is nitrified) from 0.7 at present to 0.5 would lead to an increase in dissolved inorganic nitrogen flux of about 200 t y^{-1} , but only a 3% decline in the amount of nitrogen denitrified. However, a decline in D_{\max} to 0.3 would reflect a 57% reduction in denitrification, and a dissolved inorganic nitrogen return to the water column of 1160 t y^{-1} . Therefore, a Bay-wide 50% reduction in denitrification would lead to an increased nitrogen flux similar to the input reductions currently being pursued ($\sim 1,000 \text{ t y}^{-1}$). Such a change would accompany an increase in sediment respiration of 220%. These changes are all much less than that predicted by the PPBES model to lead to catastrophic deterioration in water quality (Murray and Parslow 1996, p 101). Naturally changes in inputs will have a larger impact on a regional scale than on a Bay-wide scale. We should therefore be aiming to detect changes somewhat smaller than those listed above. Choosing 80% as the level of certainty with which we want to detect change, and four as the most replicates we could afford to collect, the level of detectable change varied both with site and flux (Table 1). Denitrification efficiency appears to be the indicator for which the smallest proportional change will be detectable, but only at the Hobsons Bay and central Port Phillip Bay sites. Temporal variation in fluxes at the Werribee coast site was so large that it would not be possible to detect there the changes in denitrification efficiency that would be expected as a result of the 1,000 t N reduction.

Table 1. Percentage level of change detectable with four replicate fluxes (at 80% certainty) using a simple t-test to compare data from different periods.

Site	Flux	Percentage change in flux	Percentage change equivalent to 1,000 t N input
Werribee	Dissolved oxygen	60	220
Werribee	Carbon dioxide	90	220
Werribee	Ammonium	>200	83
Werribee	Denitrification efficiency	70	50
Hobsons Bay	Dissolved oxygen	20	220
Hobsons Bay	Carbon dioxide	60	220
Hobsons Bay	Ammonium	80	83
Hobsons Bay	Denitrification efficiency	20	50
Central PPB	Dissolved oxygen	70	220
Central PPB	Carbon dioxide	20	220
Central PPB	Ammonium	140	83
Central PPB	Denitrification efficiency	30	50

Following the above power analysis, the recommended benthic flux sampling strategy in Longmore (2000) should be changed. The new recommendation is to measure fluxes at only two sites (Hobsons Bay and central Port Phillip Bay), with four replicate measurements per site. Sites should be sampled twice per year, in spring (after peak spring river flow) and in late summer, at the time of maximum phytoplankton production. To reduce costs, fluxes in Hobsons Bay (site 16) could be measured using an existing diver-operated chamber array, while fluxes in central Port Phillip Bay could be measured by deploying a pair of automated chambers twice. A modified costing from that

provided by Longmore (2000) is appended (Appendix 3), for estimating fluxes of oxygen, carbon dioxide, inorganic nitrogen and N₂ gas. Denitrification efficiency is calculated from the fluxes.

The statistical test for change to be applied to such data would be a simple one-way analysis of variance (ANOVA), comparing measurements from each site from one year to another. Such an analysis is capable of detecting both differences between sequential years, and over the duration of sampling. Once several years of data have accumulated, trend analysis may be more appropriate, but we do not yet have enough information to estimate levels of temporal variation and how strong important trends may be (and therefore the power needed to detect them). Also, the recommended sampling scheme is based on the assumption of event-driven changes to the Bay, rather than a gradual trend. Rysgaard *et al.* (1998) found that changes in nitrogen cycling brought about by a pulse input may disappear within a month of the input, and the PPBES model predicts that increased Yarra River loads will lead to an increase in the duration of impacts, rather than an increase in severity. We are therefore unsure that brief impacts on benthic fluxes (eg from a Yarra flood) could be detected without unrealistically high sampling rates (at least twice per month). This could be verified by sampling four times over two months at one site in Hobsons Bay after a spring flood, the results of which would guide all future sampling. This is included as a pilot study (below). Alternatively, the continuous fluorescence monitoring recommended as part of the water column monitoring proposal will provide evidence, over time, of an increase in duration of plankton blooms. Analysis of this data may indicate whether benthic flux sampling times or frequency should be changed.

Pilot studies

Pilot studies were proposed by Longmore (2000) to:

1. Compare fluxes estimated by benthic chamber to those estimated by laboratory incubation of sediment cores;
2. Determine whether nitrate or silicate pore water concentrations are adequate surrogates for denitrification efficiency;
3. Determine the impact of exotic species on denitrification;
4. Determine the impact of existing native fauna on denitrification;
5. Determine the appropriate period in which to sample fluxes.

The intent of the first two pilot studies is to determine if there are cheaper ways of determining denitrification efficiency, which could allow more spatially intensive denitrification efficiency estimates in key areas such as Hobsons Bay. The third and fourth pilot studies explore the potential of processes other than nutrient input to impact on nitrogen recycling. The need for the fifth pilot study was outlined in the previous section.

There are few published direct comparisons of benthic flux estimates from chambers with those from sediment core incubations. However, because benthic chambers often cover 5-10 times more area of sediment than cores, it is reasonable to expect that fluxes estimated from cores would be more variable than those from chambers. Miller-Wayt *et al.* (1994) found that fluxes from 12 sediment cores were needed to provide a similar standard error to that for four benthic chambers. Duplicate cores are the minimum necessary to calculate power to detect change, and the pilot study should proceed on the

basis that the appropriate number of cores needed to measure a change in denitrification efficiency will be estimated by power analysis at the end of the study.

Longmore (2000) recommended comparison of *in situ* and laboratory flux measurements, and pore water nitrate and silicate concentrations, at three sites quarterly for two years, but in light of the recommendation in this report to measure benthic fluxes at only two sites twice per year, to achieve the same number of comparisons at no extra cost will require a longer period of sampling (8 years). Longmore's (2000) recommended sample numbers were based on a "best guess", rather than a statistical analysis. There are insufficient data to enable statistical analysis of the strength of the potential relationship between nitrate or silicate concentration in sediment and denitrification efficiency. Once again, the number of samples needed to detect change, and the most appropriate statistical test, will be estimated at the completion of the pilot study. Given that one of the major benefits of a pilot study is to guide future sampling, it would be most effective to complete the pilot studies early in the program (eg. after two years). One possibility is to double the frequency of benthic flux sampling for the first two years of the program, in order to allow comparison with a suitable number of sediment pore water and sediment core incubations. The extra cost of this approach is given in Appendix 3, Table A3b. It could be argued that the number of comparative measurements included in Table A3b (2 sites x 4 chambers/cores x 4 quarters x 2 years = 64) is excessive, but until the comparisons are carried out, we have no way of knowing if this is true. If we wish to adopt a surrogate measurement of denitrification efficiency, we must first be confident of its statistical relationship with denitrification efficiency. To develop a robust relationship, we need to ensure that comparisons are made over as broad a range of measurements as we are likely to encounter.

A pilot study was also recommended to improve our understanding of the impact of exotic species on denitrification. This would involve the measurement of changes in denitrification efficiency over a range of exotic infaunal densities, by replicated flux measurements in experimentally manipulated mesocosms. No experimental data exists which could guide experimental design. Selection of type and density of species should be based on an assessment of the species most likely to impact from those currently occurring in Port Phillip Bay. A minimum of four replicate fluxes per treatment, and four treatment levels, is proposed. The presence or absence of an impact of treatment level on denitrification efficiency will be determined by simple Analysis of Variance, while strength of the relationship between treatment level and efficiency will be determined by regression.

A similar (field-based) pilot study is proposed to allow an estimate to be made of the impact of native infauna on denitrification. It is proposed that the sediment under each benthic chamber be sampled for infauna, with the aim of deriving a statistical relationship between denitrification efficiency and the number, biomass or pumping rate of infauna, so that the impact may be estimated of future changes in native infauna on denitrification. An indicative costing is presented in Appendix 3.

Water column

The water column sampling proposal included both deployment of automated monitoring systems, and the collection of extra samples in the routine EPA fixed site monitoring program. The indicators to be monitored in this approach include:

- nitrogen concentrations in the water column;
- plankton biomass;
- oxygen concentration in the water column;
- water column stratification (since it affects oxygen supply to the sediment).

The only indicators recommended by Longmore (2000) which are not currently monitored by EPA are dissolved and particulate organic nitrogen concentrations (in discrete samples) and continuous monitoring of salinity, temperature, dissolved oxygen and chlorophyll fluorescence. This proposal assumes that EPA will continue to carry the cost of the current fixed site sampling program. The cost of the extra indicators is about \$59 per site per sampling trip, while the cost of the continuous monitoring has already been estimated in Longmore (2000).

Significant design questions to which statistical advice may contribute include spatial density of sites, suitable sampling frequency and a suitable statistical analysis for resulting data. Data sets on which statistical analysis may be performed include the continuous monitoring carried out in central Port Phillip Bay by Mickelson (1990), the underway monitoring for the Port Phillip Bay Environmental Study (Longmore *et al.* 1996) and the fixed site sampling studies reported by Longmore *et al.* (1997) and Brown *et al.* (1998).

Spatial distribution of sites

CSIRO has carried out an (unpublished) analysis of variance of the underway monitoring of Port Phillip Bay (Longmore *et al.* 1996). This analysis indicated four main zones of water quality in the Bay: a central zone with small spatial gradients; a western zone (including Corio Bay), affected by the WTP discharge; a northern zone, affected by the Yarra/Maribyrnong and storm water drain runoff; and a southern zone, affected by tidal mixing. This analysis is broadly in line with the mean interpolated nutrient distributions of Shao and Fox (1996, Fig 13) and also the environmental zones defined by the Phase 1 Study (MMBW & FWD 1972). When we consider the areas likely to be impacted by changes in nutrient input, three general zones can be defined, and no further statistical analysis is necessary. Sampling should occur in the northern impact zone, the western impact zone, and in the bay centre (though isolated from inputs, it holds about 70% of the bay volume). Choice of sites within the impact zones is more difficult. Sites very close to the inputs may not show the full impact (eg. because algal blooms have not had the time to develop before water moves away from the site), whereas those further away may show the impact, but on a “noisier” background making it more difficult to detect. There are no strong reasons why sites should not be those sampled by EPA in Hobsons Bay, central Port Phillip Bay and off the Werribee coast, particularly given the time series of data already available for those sites. EPA also samples one other site, in Corio Bay, and deploying instruments at this site may be a valuable future addition to the monitoring program.

Sampling frequency, replication and power to detect change

Automated instruments are capable of sampling at very high frequencies, and the frequency finally used is often a compromise between electronic storage capacity, power supply and speed of biological fouling of sensors. The modern instruments deployed in this proposal would sample at rates far higher than any significant physical or biological process we may be interested in.

Longmore *et al.* (1997) sampled 6 sites 195 times at fortnightly intervals during 1990-97. Three of these sites approximate the EPA sites in central Port Phillip Bay/Hobsons Bay and off the WTP. Analysis of variance of ammonium, nitrite, nitrate, particulate nitrogen and chlorophyll *a* concentrations from these sites indicated significant differences between sites and years, and significant seasonal variance for all indicators except chlorophyll *a* concentration. Murray and Parslow (1997, Table 8.5) estimate that a bay-wide decline in denitrification of about 30% would be accompanied by increases in chlorophyll *a*, particulate nitrogen, dissolved organic nitrogen and ammonium concentrations of 28%, 28%, 16% and 23% respectively, and Bay-wide decreases of oxidised nitrogen and phosphate concentrations of 19% and 8% respectively. A decline in denitrification of about 57% is predicted to be accompanied by Bay-wide increases of chlorophyll *a*, particulate nitrogen, ammonium and dissolved organic nitrogen concentrations of 98%, 98%, 63% and 52% respectively, and Bay-wide decreases of oxidised nitrogen and phosphate concentrations of 11% and 2% respectively.

The number of samples required to detect a 10-200% change in a variable at 0.05 significance level (the power) was calculated for the above indicators from the three sites (Appendix 2) based on using a simple t-test approach to compare samples from different periods. When compared to the levels of change we may wish to detect and assuming 12 samples are collected each year from each site (to take advantage of the sampling already carried out monthly by EPA), it is clear that the power to detect change varies with nutrient and between sites (Table 2). Chlorophyll *a* concentration has highest power as an indicator in Hobsons Bay, but not at the other two sites. Particulate N concentration is a moderately powerful indicator at all sites. Ammonium concentration is a marginally useful indicator in Hobsons Bay and central Port Phillip Bay, but not off Werribee. Oxidised N and phosphate concentrations are not powerful indicators at any site. As with the benthic flux data, where significant seasonal variation occurs (which ANOVA indicates is the case for all of the indicators except chlorophyll *a*), ANOVA comparing means for the same season from different years should be capable of detecting smaller changes than those indicated in Table 2. Unfortunately, little seasonality was detected in the most sensitive indicator (chlorophyll *a*), so that there is no certainty that an increase in power to detect change in chlorophyll will follow from attempts to correct for seasonality.

Statistical analysis over longer time series

Continuously measured variables are best interpreted using time-series analysis, such as auto-regressive moving average (ARMA) procedures portrayed by Mickelson (1990), in which the impact of correlated factors (eg river flow, winds, evaporation) may be taken into account. Time series analysis can be used to determine trends, but is much more useful at indicating the possible cause(s) for deviation from a trend, and for providing forecasts.

As outlined above, simple t-tests could be used to compare annual average values for the nutrient and chlorophyll data, but such comparisons lack power for many of the site/nutrient combinations. More sophisticated analyses are possible when a long series of data have been collected. Goudey and Lloyd-Smith (1999) have outlined a number of methods to statistically analyse water quality data. Depending on the objective of the analysis, these include both parametric and non-parametric tests, including t-tests, analysis of variance and contingency table tests. However, the ultimate aim of each of the tests was to identify compliance with water quality objectives, without identifying any causal factors.

Table 2. Change detectable at each site (at 0.05 level of significance with 80% certainty) for 12 samples using a simple t-test to compare data from different samples.

Site	Variable	% change detectable for 12 samples	No. of samples needed to detect change equivalent to 1,000 t N input (Baywide)
Hobsons Bay	Chlorophyll <i>a</i>	12	2
	Ammonium	85	>20
	Oxidised N	140	>>20
	Particulate N	55	5
	Phosphate	22	>>20
Werribee coast	Chlorophyll <i>a</i>	90	10
	Ammonium	185	>20
	Oxidised N	185	>>20
	Particulate N	68	7
	Phosphate	44	>>20
Central PPB	Chlorophyll <i>a</i>	115	16
	Ammonium	66	11
	Oxidised N	>200	>>20
	Particulate N	40	3
	Phosphate	18	>20

The Victorian Water Quality Monitoring Network applies a Generalised Additive Model (GAM) approach to identify the magnitude and statistical significance of time trends in river samples collected over the past 10-20 years (Smith and Nathan 2000). The GAM approach corrects for missing data, serial correlation, seasonality, climatic influences (including river flow), and non-constant variance.

EPA applied a linear regression modelling technique to the fixed site information from Port Phillip Bay (Brown *et al.* 1998), by which variation of a measure could be described in terms of time and other explanatory variables. In particular, attempts were made to remove variation attributable to “natural” processes (season, river flow, temperature), so that anthropogenic impact could be identified. This is a very similar approach to the GAM procedure. Outliers and serial correlation were identified, and corrections made where necessary.

Most of the fixed site data discussed above have been collected by EPA since the start of the EPA Fixed Site Monitoring Program (in 1986), and could be analysed by either of the above approaches, with the latter approach requiring more effort, but producing much more information. While dissolved organic nitrogen and particulate nitrogen have not been measured as part of the EPA network, they were measured by Longmore *et al.* (1997) at six sites fortnightly for seven years.

EPA (1996) provided the theoretical basis on which the statistical power to detect a linear trend in a time series could be estimated. According to theory, a monotonic linear trend equivalent to a change of 1.3 standard errors of the mean could be detected with 80% power after five years of monthly sampling. When applied to the standard errors calculated for the Port Phillip Bay data collected by Longmore *et al.* (1997), very small percentage changes should be detectable for some nutrients, particularly dissolved organic nitrogen and particulate nitrogen (Table 3). Because of the higher number of samples involved, the level of change detectable by trend analysis is much smaller than that detected by t-tests between annual means (as in Table 2). Trend analysis when 5 years of data are available should easily detect the changes in chlorophyll *a*, particulate nitrogen and dissolved organic nitrogen concentrations predicted by the PPBES model for a 30% decline in denitrification, and may also barely detect the predicted changes in ammonium and phosphate concentration. However, it probably will not detect the predicted change in oxidised nitrogen concentration.

Table 3. Percentage of change that could be detected by linear trend analysis (at 0.05 level of significance with 80% power) with monthly sampling for five years, compared to model prediction of changes arising from a bay-wide 30% decline in denitrification.

Indicator	Hobsons Bay	Werribee coast	Central PPB	Bay-wide change predicted by PPBES model
Chlorophyll <i>a</i>	9%	9%	8%	+28%
Ammonium	7%	17%	9%	+23%
Oxidised nitrogen	12%	16%	23%	-19%
Particulate nitrogen	5%	6%	3%	+28%
Dissolved organic nitrogen	2%	2%	1%	+16%
Phosphate	2%	4%	1%	-8%

QUALITY ASSURANCE / QUALITY CONTROL

QA/QC procedures are designed to ensure that accurate techniques are consistently applied to samples, from their collection, through storage/preservation, eventual chemical analysis, calculation, storage of results and subsequent statistical treatment. This is important both for immediate use of the data, and for historical comparisons. Errors or inconsistent treatment at any stage compromises the data, and conclusions that may be drawn from it. Quality assurance is based on strictly adhering to documented procedures, while quality control depends primarily on comparison of analyses with other laboratories. Particular care should be taken to record any changes in field or laboratory procedures that may impact on the analytical result. Wherever possible, new procedures should be run in parallel with old procedures for sufficient time to allow an estimate of the impact of any change.

1. Field

Benthic chambers

Preparation, deployment, recovery and extraction of samples from benthic chambers should all follow a written protocol, which includes a check-list to be filled out for each deployment. Records should be kept of site location, depth, and the sample site described either from diver observation or lowering of video camera. Visual observation of the seal between chamber and sediment surface should be confirmed by injection of appropriate chemical tracer. Salinity/temperature/dissolved oxygen sensors should be maintained and calibrated as described below. Sample storage loops (on automated chambers) or syringes (on manually-sampled chambers) should be routinely cleaned and stored in seawater. Care should be taken to exclude air from samples collected for pH, alkalinity and nitrogen gas samples. Samples for all analyses, except pH and nitrogen gas, should be gently filtered through a sterile 0.45 µm membrane filter.

Any diving activities should occur according to prescribed diving safety standards (eg. draft Australian Standard for Scientific Diving). Similarly, all activities should comply with the Occupational Health and Safety Act 1985 and subsequent Regulations.

Water column sampling

Water column samples may be collected by submersible pump or Niskin bottle. In both cases, care should be taken to maintain clean samplers, and to ensure that samplers are well flushed by sample. Pumps should not be used if they affect dissolved gas distribution in the sample (eg. if cavitation produces air bubbles). The aim should be to collect a sample that represents the whole water column. Samples should be extracted from samplers using clean handling procedures (gloves), and stored in appropriately cleaned plastic bottles or Whirlpak bags. Chlorophyll and particulate N samples should be collected on GF/F filters, then stored frozen. Samples for nutrient analysis should be frozen as rapidly as possible. Waterproof field sheets are used to record site and sample information.

At least once per year, 10 replicate samples should be collected from the one site, and analysed to determine the sum of sampling, sample storage and analytical errors, and to confirm the detection limit.

Electronic equipment should be calibrated at least every month. Salinity sensors should be calibrated to better than 0.01 salinity units with Standard Seawater, and check samples collected in the field for high-precision laboratory analysis. Temperature should be calibrated against a certified thermometer (eg. SIS RTM 4002 or equivalent) to at least 0.02 °C. Dissolved oxygen sensors should be calibrated before each deployment in vapour-saturated air, but calibrations should always be checked against field samples measured in the lab by Winkler titration. Comparison of the continuous electronic records with field samples will be essential to compensate for the effect of biological fouling during deployment. Fluorometer response should be checked with water samples of known chlorophyll concentration.

2. Laboratory analysis

Chemical analyses should be carried out by a laboratory certified by the National Association of Testing Authorities (NATA) for the specific analyses. All such laboratories will have well-defined QA/QC procedures in place, which include regular inter-laboratory calibration exercises. However, the precision required for several of the measurements (salinity, alkalinity, nitrogen gas) exceeds that available in many laboratories, and a general guide to appropriate methods follows.

Salinity

Check samples for salinity should be measured in a high-precision thermostatted system, such as Guildline Autosal TM 8400B or equivalent, capable of measuring salinity to 0.001 units. The system should be calibrated using IAPSO Standard Sea Water.

Dissolved oxygen

Dissolved oxygen should be determined by Winkler titration, with spectrophotometric or redox detection of endpoint (eg. Parsons *et al.* 1984). Appropriate care should be taken throughout the procedure to prevent oxygen contamination before fixing, and loss of iodine after acidification.

Reagent blanks should be determined each time reagents are refreshed. The ultimate accuracy of the method depends on the iodate standard, which should be prepared freshly each day. Samples should be titrated in duplicate.

Chlorophyll *a*

Chlorophyll analysis is based on the extraction of pigment from glass fibre (GF/F) filters with 90% acetone, and subsequent spectrophotometric determination of pigment concentration (Strickland and Parsons 1972) using the equations of Jeffrey and Humphrey (1975).

There are no calibration standards for chlorophyll; the equations currently used were derived empirically. Accuracy ultimately depends on wavelength and absorbance, both of which should be regularly checked with standard solutions. An internal quality control sample should be prepared and analysed, along with a blank filter, with each batch of samples. A quality control chart should be used to compare internal QC samples, and the results for a batch rejected if the QC sample deviates from the chart by more than the

detection limit. External QC samples (eg. SPEX chlorophyll sample, NATA inter-laboratory samples) should be analysed annually, and the results recorded in a QC book. Chromatographic methods are now available, which provide a much more detailed measure of the range of pigments in a sample. Such methods should be adopted where cost and equipment availability make it possible.

The detection limit for chlorophyll a should be $0.1 \mu\text{g L}^{-1}$ or better.

Nutrients

Nutrient analysis may be carried out by segmented flow colorimetry, using methods developed for detection of low levels in seawater. The following methods used at MAFRI are recommended. Ammonium determination is based on the Berthelot reaction (Technicon 1973b), with corrections made for background colour, turbidity and salinity. Detection limit is better than $0.05 \mu\text{M}$. Nitrite is determined by the method of Bendschneider and Robinson (1952) as automated by Technicon (1972), with a detection limit of $0.01 \mu\text{M}$. Nitrate is determined by the method of Morris and Riley (1963) as modified by Strickland and Parsons (1972) and automated by Technicon (1972), with a detection limit of $0.02 \mu\text{M}$. Phosphate is determined by the method of Murphy and Riley (1963) as modified by Strickland and Parsons (1972) and automated by Technicon (1973c), with a detection limit of $0.06 \mu\text{M}$. Silicate is analysed by the method of Koroleff (1972) as automated by Technicon (1972), with a detection limit of $0.1 \mu\text{M}$. Particulate N is determined by digestion of filter (Nicholls 1975). Kjeldahl nitrogen is determined by the same method on a unfiltered water sample, and dissolved organic nitrogen calculated as (Kjeldahl N - ammonium - particulate N). Detection limits are $0.2 \mu\text{M}$ for Kjeldahl N, $0.02 \mu\text{M}$ particulate N and $0.25 \mu\text{M}$ dissolved organic N.

Samples are always analysed in duplicate. If duplicates differ by more than twice the detection limit, the analysis are repeated. QC procedures include daily analysis of two different internally-generated QC samples, and regular analysis of Low-Level Nutrient workshop inter-laboratory calibration samples and NATA inter-laboratory calibration samples. Blanks and Standard Reference Material (NRC-CNRC BCSS-1 or PACS-1) are analysed with each batch for particulate N. A log book is maintained to record reagent and standard preparation, maintenance operations and comparison of analysis of daily standards and internal QC samples. A quality control chart is used to compare internal QC samples, and the results for a batch rejected if the QC sample deviates from the chart by more than the detection limit.

pH, Alkalinity, total carbon dioxide

The precision required for pH, alkalinity and total carbon dioxide analyses exceeds that of standard methods (eg. APHA 1992). The following methods should therefore be followed.

The system used to measure pH needs to be capable of sensing a change of 0.001 pH units. This requires measurement in a water bath at 25°C , using a meter and electrode calibrated with high-accuracy pH buffers (eg. Cole Parmer pH 4.000 and 7.000). Electrode response is measured before and after each batch of samples, and the electrode is conditioned to the salinity of samples before measurement begins.

Alkalinity is determined by a modification of the Gran titration, in which sample is titrated in a thermostatically controlled vessel at 25°C with standardised weak HCl to a pH of 3.5 (Dickson 1981). Electrodes are calibrated to within ± 0.002 pH units, and corrections are made for electrode drift. Filtered (0.45 μm) samples are analysed as soon as possible after collection, but are stable for weeks when stored air-tight at 4°C. The impact of varying sample salinity on electrode potential is overcome by titrating 4 mL of sample in 20 mL of artificial seawater. At least four blanks are titrated at the start of each day to ensure reliable operation of electrodes and burette. Total carbon dioxide concentration is calculated from pH, alkalinity, temperature and salinity, to a precision of better than 6 μM .

QC procedures include corrections of pH electrode response for drift, and multiple measurement of an internal QC sample during a batch. A standard reference material for alkalinity and total carbon dioxide concentrations (University of California San Diego) is run after each six samples, and every tenth sample is analysed in duplicate. Groups of six samples are re-analysed if the reference samples before and after differ by more than twice the precision.

Nitrogen gas

We need to be able to detect changes in nitrogen concentration as small as 0.5 μM , on a background of about 450 μM . This requires particular care during both sampling and analysis. Samples collected from chambers are stored in gas tight glass stoppered test tubes, poisoned with mercuric chloride. N₂/Ar ratios in the samples are measured by a membrane inlet mass spectrometer (MIMS) using the methods of Kanat *et al* (1994).

Standards consist of air equilibrated water maintained at a constant temperature and salinity (0, 3, 6, 10, 20 and 30 psu) for > 12 hours. Each flask is loosely stoppered to maintain 100% relative humidity. N₂/Ar ratios in the standards are determined from equations derived by Weiss (1970). Absolute N₂ concentration in the samples is estimated using the salinity and temperature of the chamber water and assuming atmospheric equilibrium. The CV of N₂/Ar = 0.04%.

3. Data management

After data entry by one person, and independent checking of the entry by another person, field sheets are permanently archived. Field data and chemical analyses are merged in the one database, after which statistical checks on the data are run. These may include a comparison of the measurement with summary statistics for the site, to flag possible errors during data entry or analysis. Data which require further manipulation (eg calculation of benthic fluxes) are extracted into a subsidiary (temporary) database.

Calculation of fluxes

Fluxes are calculated by linear regression of sample concentration on time. Analytical errors, regression errors and chamber volume errors are all carried through the calculations to provide an overall estimate of precision. Replicate fluxes are compared, and if fluxes differ significantly, all earlier steps are checked again. All final results are stored in a third (permanent) database.

Data storage

All information should be stored electronically in a form readily exported to common software platforms. Data should be backed up as often as necessary to ensure no permanent loss occurs as a result of system break-down.

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Hobsons Bay

Oxi di sed N

		Percentage change to be detected																			
		10	20	30	40	50	60	70	80	90	100	110	120	130	140	150	160	170	180	190	200
Replicates	per site																				
2		0.05	0.05	0.05	0.06	0.06	0.06	0.07	0.07	0.08	0.08	0.09	0.10	0.11	0.12	0.13	0.14	0.15	0.16	0.17	0.18
3		0.05	0.05	0.06	0.06	0.07	0.08	0.09	0.10	0.12	0.13	0.15	0.17	0.19	0.21	0.23	0.26	0.29	0.31	0.34	0.37
4		0.05	0.06	0.06	0.07	0.08	0.10	0.11	0.13	0.15	0.18	0.21	0.24	0.27	0.30	0.34	0.38	0.42	0.46	0.50	0.54
5		0.05	0.06	0.07	0.08	0.09	0.11	0.14	0.16	0.19	0.23	0.26	0.31	0.35	0.39	0.44	0.49	0.54	0.58	0.63	0.67
6		0.05	0.06	0.07	0.08	0.10	0.13	0.16	0.19	0.23	0.27	0.32	0.37	0.42	0.48	0.53	0.58	0.63	0.68	0.73	0.77
7		0.05	0.06	0.07	0.09	0.12	0.15	0.18	0.22	0.27	0.32	0.38	0.43	0.49	0.55	0.61	0.66	0.72	0.76	0.81	0.85
8		0.05	0.06	0.08	0.10	0.13	0.16	0.20	0.25	0.31	0.37	0.43	0.49	0.55	0.62	0.68	0.73	0.78	0.83	0.86	0.90
9		0.05	0.06	0.08	0.11	0.14	0.18	0.23	0.28	0.34	0.41	0.48	0.55	0.61	0.68	0.74	0.79	0.84	0.87	0.91	0.93
10		0.05	0.07	0.09	0.11	0.15	0.20	0.25	0.31	0.38	0.45	0.52	0.60	0.66	0.73	0.79	0.83	0.88	0.91	0.94	0.96
11		0.05	0.07	0.09	0.12	0.16	0.21	0.27	0.34	0.41	0.49	0.57	0.64	0.71	0.77	0.83	0.87	0.91	0.94	0.96	0.97
12		0.05	0.07	0.09	0.13	0.17	0.23	0.30	0.37	0.45	0.53	0.61	0.68	0.75	0.81	0.86	0.90	0.93	0.95	0.97	0.98
13		0.06	0.07	0.10	0.14	0.19	0.25	0.32	0.40	0.48	0.56	0.65	0.72	0.79	0.84	0.89	0.92	0.95	0.97	0.98	0.99
14		0.06	0.07	0.10	0.14	0.20	0.26	0.34	0.42	0.51	0.60	0.68	0.75	0.82	0.87	0.91	0.94	0.96	0.98	0.99	0.99
15		0.06	0.07	0.11	0.15	0.21	0.28	0.36	0.45	0.54	0.63	0.71	0.79	0.85	0.89	0.93	0.96	0.97	0.98	0.99	1.00
16		0.06	0.08	0.11	0.16	0.22	0.30	0.38	0.48	0.57	0.66	0.74	0.81	0.87	0.91	0.94	0.97	0.98	0.99	0.99	1.00
17		0.06	0.08	0.11	0.17	0.23	0.31	0.40	0.50	0.60	0.69	0.77	0.84	0.89	0.93	0.96	0.97	0.99	0.99	1.00	1.00
18		0.06	0.08	0.12	0.17	0.24	0.33	0.42	0.52	0.62	0.71	0.79	0.86	0.91	0.94	0.97	0.98	0.99	1.00	1.00	1.00
19		0.06	0.08	0.12	0.18	0.26	0.34	0.44	0.55	0.65	0.74	0.82	0.88	0.92	0.95	0.97	0.99	0.99	1.00	1.00	1.00
20		0.06	0.08	0.13	0.19	0.27	0.36	0.46	0.57	0.67	0.76	0.84	0.89	0.93	0.96	0.98	0.99	1.00	1.00	1.00	1.00

Werribee coast

Oxi di sed N

		Percentage change to be detected																			
		10	20	30	40	50	60	70	80	90	100	110	120	130	140	150	160	170	180	190	200
Replicates per site																					
2		0.05	0.05	0.05	0.05	0.05	0.06	0.06	0.06	0.06	0.07	0.07	0.08	0.08	0.09	0.09	0.10	0.10	0.11	0.11	0.12
3		0.05	0.05	0.05	0.06	0.06	0.07	0.07	0.08	0.08	0.09	0.10	0.11	0.12	0.14	0.15	0.16	0.18	0.19	0.21	0.22
4		0.05	0.05	0.06	0.06	0.07	0.07	0.08	0.09	0.11	0.12	0.13	0.15	0.17	0.19	0.21	0.23	0.25	0.27	0.30	0.33
5		0.05	0.05	0.06	0.06	0.07	0.08	0.10	0.11	0.13	0.14	0.16	0.19	0.21	0.24	0.26	0.29	0.32	0.35	0.39	0.42
6		0.05	0.05	0.06	0.07	0.08	0.09	0.11	0.13	0.15	0.17	0.19	0.22	0.25	0.28	0.32	0.35	0.39	0.43	0.47	0.51
7		0.05	0.06	0.06	0.07	0.08	0.10	0.12	0.14	0.17	0.19	0.22	0.26	0.29	0.33	0.37	0.41	0.46	0.50	0.54	0.59
8		0.05	0.06	0.06	0.08	0.09	0.11	0.13	0.16	0.19	0.22	0.25	0.29	0.34	0.38	0.42	0.47	0.52	0.56	0.61	0.65
9		0.05	0.06	0.07	0.08	0.10	0.12	0.14	0.17	0.21	0.24	0.28	0.33	0.38	0.42	0.47	0.52	0.57	0.62	0.67	0.71
10		0.05	0.06	0.07	0.08	0.10	0.13	0.16	0.19	0.23	0.27	0.31	0.36	0.41	0.47	0.52	0.57	0.62	0.67	0.72	0.76
11		0.05	0.06	0.07	0.09	0.11	0.14	0.17	0.20	0.25	0.29	0.34	0.40	0.45	0.51	0.56	0.62	0.67	0.72	0.77	0.81
12		0.05	0.06	0.07	0.09	0.12	0.14	0.18	0.22	0.27	0.32	0.37	0.43	0.49	0.55	0.60	0.66	0.71	0.76	0.80	0.84
13		0.05	0.06	0.08	0.10	0.12	0.15	0.19	0.24	0.29	0.34	0.40	0.46	0.52	0.58	0.64	0.70	0.75	0.80	0.84	0.87
14		0.05	0.06	0.08	0.10	0.13	0.16	0.20	0.25	0.31	0.36	0.43	0.49	0.55	0.62	0.68	0.73	0.78	0.83	0.86	0.90
15		0.05	0.06	0.08	0.10	0.13	0.17	0.22	0.27	0.33	0.39	0.45	0.52	0.59	0.65	0.71	0.76	0.81	0.85	0.89	0.92
16		0.05	0.06	0.08	0.11	0.14	0.18	0.23	0.28	0.34	0.41	0.48	0.55	0.62	0.68	0.74	0.79	0.84	0.88	0.91	0.93
17		0.05	0.06	0.08	0.11	0.15	0.19	0.24	0.30	0.36	0.43	0.50	0.57	0.64	0.71	0.77	0.82	0.86	0.90	0.93	0.95
18		0.05	0.07	0.09	0.11	0.15	0.20	0.25	0.31	0.38	0.45	0.53	0.60	0.67	0.73	0.79	0.84	0.88	0.91	0.94	0.96
19		0.05	0.07	0.09	0.12	0.16	0.21	0.26	0.33	0.40	0.48	0.55	0.63	0.69	0.76	0.81	0.86	0.90	0.93	0.95	0.97
20		0.05	0.07	0.09	0.12	0.16	0.22	0.28	0.35	0.42	0.50	0.57	0.65	0.72	0.78	0.83	0.88	0.91	0.94	0.96	0.97

Werribee coast

Ammonium

		Percentage change to be detected																			
		10	20	30	40	50	60	70	80	90	100	110	120	130	140	150	160	170	180	190	200
Replicates per site																					
2		0.05	0.05	0.05	0.05	0.05	0.06	0.06	0.06	0.07	0.07	0.07	0.08	0.08	0.09	0.09	0.10	0.10	0.11	0.12	0.12
3		0.05	0.05	0.05	0.06	0.06	0.07	0.07	0.08	0.09	0.09	0.10	0.11	0.13	0.14	0.15	0.16	0.18	0.19	0.21	0.23
4		0.05	0.05	0.06	0.06	0.07	0.07	0.08	0.09	0.11	0.12	0.13	0.15	0.17	0.19	0.21	0.23	0.26	0.28	0.31	0.33
5		0.05	0.05	0.06	0.06	0.07	0.08	0.10	0.11	0.13	0.15	0.17	0.19	0.21	0.24	0.27	0.30	0.33	0.36	0.40	0.43
6		0.05	0.05	0.06	0.07	0.08	0.09	0.11	0.13	0.15	0.17	0.20	0.23	0.26	0.29	0.32	0.36	0.40	0.44	0.48	0.52
7		0.05	0.06	0.06	0.07	0.09	0.10	0.12	0.14	0.17	0.20	0.23	0.26	0.30	0.34	0.38	0.42	0.47	0.51	0.55	0.60
8		0.05	0.06	0.06	0.08	0.09	0.11	0.13	0.16	0.19	0.22	0.26	0.30	0.34	0.39	0.43	0.48	0.53	0.57	0.62	0.66
9		0.05	0.06	0.07	0.08	0.10	0.12	0.15	0.18	0.21	0.25	0.29	0.33	0.38	0.43	0.48	0.53	0.58	0.63	0.68	0.72
10		0.05	0.06	0.07	0.08	0.10	0.13	0.16	0.19	0.23	0.27	0.32	0.37	0.42	0.48	0.53	0.58	0.63	0.68	0.73	0.77
11		0.05	0.06	0.07	0.09	0.11	0.14	0.17	0.21	0.25	0.30	0.35	0.40	0.46	0.52	0.57	0.63	0.68	0.73	0.77	0.82
12		0.05	0.06	0.07	0.09	0.12	0.15	0.18	0.22	0.27	0.32	0.38	0.44	0.50	0.56	0.61	0.67	0.72	0.77	0.81	0.85
13		0.05	0.06	0.08	0.10	0.12	0.16	0.20	0.24	0.29	0.35	0.41	0.47	0.53	0.59	0.65	0.71	0.76	0.80	0.85	0.88
14		0.05	0.06	0.08	0.10	0.13	0.17	0.21	0.26	0.31	0.37	0.43	0.50	0.56	0.63	0.69	0.74	0.79	0.84	0.87	0.90
15		0.05	0.06	0.08	0.10	0.14	0.17	0.22	0.27	0.33	0.40	0.46	0.53	0.60	0.66	0.72	0.77	0.82	0.86	0.90	0.92
16		0.05	0.06	0.08	0.11	0.14	0.18	0.23	0.29	0.35	0.42	0.49	0.56	0.63	0.69	0.75	0.80	0.85	0.88	0.91	0.94
17		0.05	0.07	0.08	0.11	0.15	0.19	0.25	0.30	0.37	0.44	0.51	0.58	0.65	0.72	0.78	0.83	0.87	0.90	0.93	0.95
18		0.05	0.07	0.09	0.12	0.15	0.20	0.26	0.32	0.39	0.46	0.54	0.61	0.68	0.74	0.80	0.85	0.89	0.92	0.94	0.96
19		0.05	0.07	0.09	0.12	0.16	0.21	0.27	0.34	0.41	0.48	0.56	0.63	0.70	0.77	0.82	0.87	0.90	0.93	0.95	0.97
20		0.05	0.07	0.09	0.12	0.17	0.22	0.28	0.35	0.43	0.51	0.58	0.66	0.73	0.79	0.84	0.88	0.92	0.94	0.96	0.98

Central Port Phillip Bay

Chlorophyll a

		Percentage change to be detected																			
		10	20	30	40	50	60	70	80	90	100	110	120	130	140	150	160	170	180	190	200
Replicates	per site																				
2		0.05	0.05	0.05	0.06	0.06	0.07	0.07	0.08	0.09	0.10	0.11	0.12	0.13	0.14	0.16	0.17	0.18	0.20	0.21	0.23
3		0.05	0.05	0.06	0.07	0.08	0.09	0.11	0.12	0.14	0.17	0.19	0.22	0.25	0.28	0.31	0.34	0.38	0.41	0.45	0.49
4		0.05	0.06	0.07	0.08	0.10	0.12	0.14	0.17	0.20	0.24	0.27	0.32	0.36	0.41	0.45	0.50	0.55	0.60	0.64	0.69
5		0.05	0.06	0.07	0.09	0.11	0.14	0.17	0.21	0.25	0.30	0.35	0.41	0.46	0.52	0.58	0.63	0.68	0.73	0.78	0.82
6		0.05	0.06	0.08	0.10	0.13	0.16	0.21	0.25	0.31	0.37	0.43	0.49	0.56	0.62	0.68	0.73	0.78	0.83	0.86	0.90
7		0.05	0.06	0.08	0.11	0.14	0.19	0.24	0.30	0.36	0.43	0.50	0.57	0.64	0.70	0.76	0.81	0.85	0.89	0.92	0.94
8		0.05	0.07	0.09	0.12	0.16	0.21	0.27	0.34	0.41	0.49	0.56	0.64	0.71	0.77	0.82	0.87	0.90	0.93	0.95	0.97
9		0.05	0.07	0.09	0.13	0.18	0.24	0.30	0.38	0.46	0.54	0.62	0.70	0.76	0.82	0.87	0.91	0.94	0.96	0.97	0.98
10		0.06	0.07	0.10	0.14	0.19	0.26	0.33	0.42	0.50	0.59	0.67	0.75	0.81	0.86	0.91	0.94	0.96	0.98	0.99	0.99
11		0.06	0.07	0.11	0.15	0.21	0.28	0.37	0.45	0.55	0.64	0.72	0.79	0.85	0.90	0.93	0.96	0.97	0.99	0.99	1.00
12		0.06	0.08	0.11	0.16	0.23	0.31	0.40	0.49	0.59	0.68	0.76	0.83	0.88	0.92	0.95	0.97	0.98	0.99	1.00	1.00
13		0.06	0.08	0.12	0.17	0.24	0.33	0.43	0.53	0.62	0.72	0.79	0.86	0.91	0.94	0.97	0.98	0.99	1.00	1.00	1.00
14		0.06	0.08	0.12	0.18	0.26	0.35	0.45	0.56	0.66	0.75	0.83	0.88	0.93	0.96	0.98	0.99	0.99	1.00	1.00	1.00
15		0.06	0.08	0.13	0.19	0.28	0.37	0.48	0.59	0.69	0.78	0.85	0.91	0.94	0.97	0.98	0.99	1.00	1.00	1.00	1.00
16		0.06	0.09	0.14	0.20	0.29	0.40	0.51	0.62	0.72	0.81	0.88	0.92	0.96	0.98	0.99	0.99	1.00	1.00	1.00	1.00
17		0.06	0.09	0.14	0.22	0.31	0.42	0.53	0.65	0.75	0.83	0.90	0.94	0.97	0.98	0.99	1.00	1.00	1.00	1.00	1.00
18		0.06	0.09	0.15	0.23	0.33	0.44	0.56	0.67	0.77	0.85	0.91	0.95	0.97	0.99	0.99	1.00	1.00	1.00	1.00	1.00
19		0.06	0.10	0.15	0.24	0.34	0.46	0.58	0.70	0.80	0.87	0.93	0.96	0.98	0.99	1.00	1.00	1.00	1.00	1.00	1.00
20		0.06	0.10	0.16	0.25	0.36	0.48	0.61	0.72	0.82	0.89	0.94	0.97	0.99	0.99	1.00	1.00	1.00	1.00	1.00	1.00

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		Percentage change to be detected																			
		10	20	30	40	50	60	70	80	90	100	110	120	130	140	150	160	170	180	190	200
Replicates	per site																				
2		0.05	0.05	0.05	0.05	0.05	0.05	0.06	0.06	0.06	0.06	0.07	0.07	0.07	0.08	0.08	0.08	0.09	0.09	0.10	0.10
3		0.05	0.05	0.05	0.06	0.06	0.06	0.07	0.07	0.08	0.08	0.09	0.10	0.10	0.11	0.12	0.13	0.14	0.15	0.17	0.18
4		0.05	0.05	0.05	0.06	0.06	0.07	0.07	0.08	0.09	0.10	0.11	0.12	0.14	0.15	0.17	0.18	0.20	0.22	0.24	0.26
5		0.05	0.05	0.06	0.06	0.07	0.07	0.08	0.09	0.11	0.12	0.13	0.15	0.17	0.19	0.21	0.23	0.25	0.28	0.30	0.33
6		0.05	0.05	0.06	0.06	0.07	0.08	0.09	0.11	0.12	0.14	0.16	0.18	0.20	0.22	0.25	0.28	0.31	0.34	0.37	0.40
7		0.05	0.05	0.06	0.07	0.08	0.09	0.10	0.12	0.14	0.16	0.18	0.20	0.23	0.26	0.29	0.32	0.36	0.39	0.43	0.47
8		0.05	0.05	0.06	0.07	0.08	0.09	0.11	0.13	0.15	0.17	0.20	0.23	0.26	0.30	0.33	0.37	0.41	0.45	0.49	0.53
9		0.05	0.06	0.06	0.07	0.08	0.10	0.12	0.14	0.16	0.19	0.22	0.26	0.29	0.33	0.37	0.41	0.46	0.50	0.54	0.58
10		0.05	0.06	0.06	0.07	0.09	0.11	0.13	0.15	0.18	0.21	0.25	0.28	0.32	0.37	0.41	0.45	0.50	0.55	0.59	0.64
11		0.05	0.06	0.07	0.08	0.09	0.11	0.14	0.16	0.19	0.23	0.27	0.31	0.35	0.40	0.45	0.49	0.54	0.59	0.64	0.68
12		0.05	0.06	0.07	0.08	0.10	0.12	0.15	0.18	0.21	0.25	0.29	0.33	0.38	0.43	0.48	0.53	0.58	0.63	0.68	0.72
13		0.05	0.06	0.07	0.08	0.10	0.13	0.15	0.19	0.22	0.27	0.31	0.36	0.41	0.46	0.52	0.57	0.62	0.67	0.72	0.76
14		0.05	0.06	0.07	0.09	0.11	0.13	0.16	0.20	0.24	0.28	0.33	0.38	0.44	0.49	0.55	0.60	0.66	0.70	0.75	0.79
15		0.05	0.06	0.07	0.09	0.11	0.14	0.17	0.21	0.25	0.30	0.35	0.41	0.47	0.52	0.58	0.64	0.69	0.74	0.78	0.82
16		0.05	0.06	0.07	0.09	0.12	0.15	0.18	0.22	0.27	0.32	0.38	0.43	0.49	0.55	0.61	0.67	0.72	0.77	0.81	0.85
17		0.05	0.06	0.07	0.09	0.12	0.15	0.19	0.23	0.28	0.34	0.40	0.46	0.52	0.58	0.64	0.69	0.75	0.79	0.83	0.87
18		0.05	0.06	0.08	0.10	0.12	0.16	0.20	0.25	0.30	0.36	0.42	0.48	0.54	0.60	0.66	0.72	0.77	0.82	0.85	0.89
19		0.05	0.06	0.08	0.10	0.13	0.17	0.21	0.26	0.31	0.37	0.44	0.50	0.57	0.63	0.69	0.74	0.79	0.84	0.87	0.90
20		0.05	0.06	0.08	0.10	0.13	0.17	0.22	0.27	0.33	0.39	0.46	0.52	0.59	0.65	0.71	0.77	0.81	0.86	0.89	0.92

APPENDIX 3. MODIFIED COSTING FOR BENTHIC FLUX ESTIMATES.

This estimate is based on the use of an existing manually-sampled benthic chamber array to provide four flux estimates in Hobsons Bay, and the use of automated chambers (one existing, one new) deployed twice over two days to estimate fluxes in central Port Phillip Bay.

Table A3a. Cost estimates for sampling benthic fluxes with a combination of manually-sampled and automated chambers, twice per year.

Item	Cost per year (2 sites, 4 fluxes per site, twice per year)
Up-front (construction of second chamber)	\$10,000
Boat (2 x 3 x 8 hrs), staff etc (2 x 3 x 8 hours), divers (2 x 2 x 8 hrs).	\$12,240
Chemical analysis (70 samples)	\$6,560
Reporting/interpretation (3 days)	\$1,500

Table A3b. Indicative cost of PILOT STUDIES 1 and 2 if completed over two years (surrogate measures of denitrification efficiency).

To complete pilot studies within a two-year period, sampling of benthic fluxes, sectioning of sediment cores for nitrate and silicate concentration and laboratory incubation of sediment cores is carried out at two sites, four times per year over two years. This involves a doubling of the benthic chamber measurements proposed above, and collection of one sediment core from under each benthic chamber. If sampling is spread out over a longer period, the costs of individual pilot studies are given in Longmore (2000).

Item	Cost for two years
Extra benthic fluxes	\$40,600
Sediment nitrate and silicate profiles	\$33,600
Laboratory core incubation	\$36,000
Reporting	\$2,400

Table A3c. Indicative cost of PILOT STUDY 3 (measuring the impact of exotic fauna on denitrification efficiency).

Assumes laboratory tanks are set up with representative native fauna in a sediment layer. Varying numbers of exotic fauna (*Sabella* and *Corbula*) would be introduced to chambers in the tanks, and changes in fluxes measured relative to chambers without exotics. Assumes four chambers at each of 4 levels of biomass, by two species (32 benthic chamber incubations).

Item	Cost
Sample collection	\$1,500
Establishing and maintaining tanks	\$2,000
Measuring benthic fluxes	\$1,200
Chemical analysis (\$830 x 32)	\$26,560
Reporting	\$2,400

Table A3d. Indicative cost of PILOT STUDY 4 (measuring the impact of native fauna on denitrification efficiency).

Based on collection of one sample per chamber, four chambers/site, two sites, twice per year for two years. Sorted to identify all species. Biomass estimated for each species.

Item	Cost	Number	Annual Cost
Consumables	\$500	1	\$ 500
Sampling	\$600/day	2	\$ 1,200
Sorting, ID, biomass	\$1,000/sample	16	\$16,000
Reporting	\$400/day	10 days	\$ 4,000
Total			\$21,700

Table A3e. Indicative cost of PILOT STUDY 5 (determining the appropriate period over which benthic fluxes should be sampled).

Based on sampling one site (Hobsons Bay) four times over two months after a major river flow. Assumes four replicate fluxes per sampling, using manual chambers.

Item	Cost (1 site, 4 fluxes per site, four times)
Boat (4x 10 hrs), staff etc (4 x 10 hours), divers (4 x 2 x 10 hrs).	\$8,950
Chemical analysis (84 samples incl N ₂)	\$8,672
Reporting/interpretation (3 days)	\$1,500