NHT FINAL REPORT

DETERMINING THE ECOLOGICAL HEALTH OF ESTUARIES IN NW TASMANIA: A CASE STUDY ASSESSING THE STATUS OF THE DUCK, MONTAGU, DETENTION AND BLACK RIVER ESTUARIES

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April 2007

NHT Project No. 52508

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DETERMINING THE ECOLOGICAL HEALTH OF ESTUARIES IN NW TASMANIA: A CASE STUDY ASSESSING THE STATUS OF THE DUCK, MONTAGU, DETENTION AND BLACK RIVER ESTUARIES

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Summary

Identifying suitable reference points is critical for effective monitoring of any natural system. Here we utilize a comparative approach where putative impacts within two estuaries are determined via comparison with two reference estuaries deemed to be relatively free of human impacts. The two putatively impacted estuaries considered in this study were the Duck and Montagu River estuaries situated on the NW coast of Tasmania. The river catchments of these estuaries are typical of the region in that their catchments have become highly modified through the clearance of native vegetation and subsequent replacement with intensive agriculture, in particular dairy farming. Nutrient loadings (nitrogen and phosphorous) for these catchments are amongst the highest in Tasmania. The catchments for the Black and Detention estuaries, by comparison, are located for the most part in State Forest with limited grazing in the lower catchment.

This study considered a range of water-column and sediment (benthos) based variables commonly used to monitor estuaries. These included: salinity, dissolved oxygen, turbidity, nutrient and chlorophyll *a* levels for the water-column; and sediment redox, organic carbon content, chlorophyll *a* and macroinvertebrate community structure amongst the benthos. In addition to comparing reference with impacted estuaries, comparisons were also made across seasons, commensurate with seasonal changes in freshwater river input, and between regions within estuaries (upper and lower reaches) - previously identified in Hirst et al. (2005). This design enabled us to examine whether the detection of impacts (i.e. differences between reference and impacted systems) was contingent on the time and location of sampling or independent of these factors. Previous work had indicated that impacts may only be manifested at certain times, and in certain regions, within these estuaries.

Whilst there was clear evidence of lower water quality in the Duck and Montagu estuaries, in terms of higher dissolved phosphorous levels, turbidity and to a lesser extent water-column based algal productivity, this translated into few discernable impacts. For example, there were no significant changes in oxygen levels, sediment chemistry or macroinvertebrate community composition. Thus, these estuaries appeared to be in reasonably good health despite the poor condition of their respective catchments. Moreover, where impacts were detected their presence was variable in space and time. Reductions in water quality, relative to the reference estuaries, tended to be 1) restricted to the upper reaches of these estuaries where tidal exchange is limited, and 2) more pronounced in spring/winter compared to summer/autumn when freshwater river inputs were higher.

The results of this study clearly reinforce that how, where and when one samples is as important as the specific indicators chosen when monitoring estuaries. Comparisons are meaningless unless made against appropriate reference points, although the identification of suitable reference points will inevitably be problematic. This study found that detectable changes in water quality were restricted to the period of high river flows (winter/spring) and invariably within the upper reaches of estuaries. Failure to sample at the appropriate spatial

and temporal scales may therefore lead to erroneous conclusions about the extent of impacts within estuaries in the NW. Given limited resources we recommend sampling in spring (following winter rainfalls) rather than in summer or autumn, with a greater emphasis placed on the upper compared to the lower reaches of an estuary. Monitoring of the lower reaches is of interest, but should not be carried-out at expense of sampling in the upper reaches. Where possible assessments should be based on >1 years data to increase certainty regarding the status of an estuaries health.

When selecting appropriate indicators, greater emphasis should be given to benthic processes and variables over water-column variables. In general these indicator variables displayed less variability than the water-column variables and the benthos is known to be an important site for the storage and processing of nutrients within estuaries. We recommend collecting information on macroinvertebrate community structure, sediment chlorophyll *a* levels and where possible carbon and nitrogen levels in the sediments. Routine monitoring of salinity, dissolved oxygen, turbidity, nutrients and water-column chlorophyll *a* should be collected where possible, but this data is inherently more difficult to interpret due to higher variability. Note that these recommendations refer to meso-tidal estuaries in the NW and do not necessarily apply to estuaries elsewhere in Tasmania where tidal ranges and river flows may be very different.

An additional component of this study investigated whether stable isotopes of nitrogen could be used as broad indicators of anthropogenically-derived sources of nitrogen enrichment in estuaries (i.e. fertilizer applied to dairy pasture). This method has been successfully applied elsewhere because anthropogenic sources of nitrogen have distinct (and elevated) isotopic ratios (signatures). Stable isotope signatures of deposit-feeding invertebrates and sediments collected from the upper reaches of these estuaries were more enriched in the impacted (Duck and Montagu) compared to the reference estuaries (Detention and Black), but this was not the case for Pacific oysters collected from the lower reaches. Consequently, while there is evidence that food-webs in the upper reaches were linked to terrestrially derived sources of nitrogen, food-webs in the lower reaches remain largely uncoupled from these sources. We attributed this effect to greater tidal flushing in the lower compared to the upper reaches. This tidal flushing plays a large part in maintaining the health and resilience of these estuaries.

Introduction

Determining the ecological health of estuaries in NW Tasmania

Crucial to all efforts to detect and quantify human impacts in natural ecosystems, be they estuaries, lakes or streams, is the need to identify suitable reference points, free of human impacts against which changes can be measured. Such reference points effectively act as scientific controls whereby all extrinsic differences, except the one under examination, are minimized across comparisons – eliminating possible alternative explanations (by negating confounding variables), reducing ambiguity and increasing certainty about the impact investigated. This is important because the cost of incorrectly assessing human impacts is potentially high in terms of misdirecting scarce resources where impacts are incorrectly inferred. Detection of human impacts is further complicated by the inherent spatial and temporal variability of natural systems leading inevitably to difficulties in distinguishing natural from anthropogenic sources of variation.

In Tasmania and elsewhere in south-eastern Australia human impacts in estuaries have a long history (i.e. dating back in many cases to the late 19th century) and are often diffuse in form. This makes identification of suitable reference points problematic on two counts. Firstly, there is little information about the condition of the estuary pre-impacts, from which to draw conclusions about the level of change. This precludes the use of standard Before-After-Control-Impact (BACI) monitoring designs. Secondly, the spatially diffuse nature of human impacts in many small to medium sized estuaries (particularly those impacts originating from within the catchment) makes it difficult to locate reference sites within estuaries that are independent of the scale of the impact.

Although not often explicitly stated, most monitoring programs aim to detect departure from what is considered be a 'healthy' state. Most would agree that estuarine ecosystems in Tasmania were healthy prior to the arrival of Europeans in the 1800s but we don't actually know what estuaries were like prior to european settlement, and hence it is difficult to ascertain to what extent many estuaries have been affected by human impacts. However, if we are to assess estuaries using an evidence-based approach, and in the process detect either improvement or deterioration in condition, we will need to define what we consider healthy.

One potential solution is to use estuaries located in minimally impacted catchments or that are largely unmodified by human activities as reference points (i.e. reference estuaries). This solution at least partially circumvents the above problems by a) locating reference sites in estuaries un-affected by impacts; and b) providing a quasi temporal control (space substituted for time) through the use of a reference point that resembles more closely the pre-impact condition of the 'impacted' estuary (i.e. assumed, but impossible to test). Naturally, the choice of reference estuaries requires careful consideration so that valid comparisons can be made. Failure to do this may lead researchers to conclude, incorrectly, that estuaries are impacted when in fact the differences may have existed from the outset. One way of minimizing this risk is to use a paired-reference estuary approach, whereby the impacted estuary is compared with not just one, but two reference estuaries. The logic is similar to that used in a MBACI (multiple control-impact design) where more than one control site is used to investigate the effects of a putative impact at the 'impacted' site (Keough and Mapstone 1995).

Use of paired spatial controls (estuaries) to monitor/assess estuarine health

The extent of human impacts in putatively 'impacted' estuaries in this study was determined using a paired-reference estuary approach illustrated in figure 1. Each impacted estuary is compared with each of the reference estuaries, which in turn are compared with one another. Significant impacts were inferred if both reference estuaries consistently differed from the 'impacted' estuary, but could not generally be distinguished from each other. The most plausible explanation for this outcome is that the differences detected between estuaries are due to the putative impacts (identified prior to the study) and not other coincidental differences that may have existed naturally between estuaries. Alternatively, if all estuaries differ from each other then any difference detected between the reference and impacted estuaries are equally likely to be due natural variability, rather than any human impact per se.

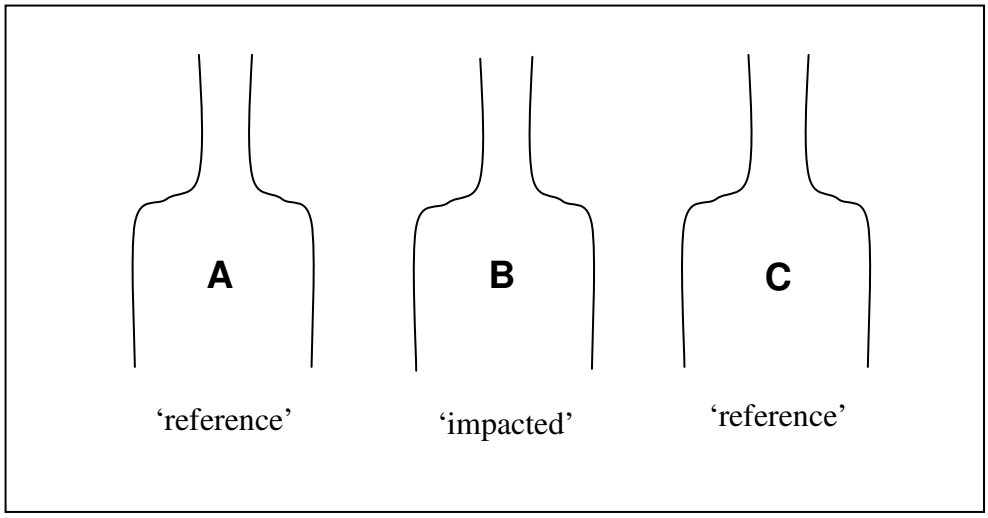

Figure 1. Paired-reference estuary approach used to assess estuaries in this study. A and C are reference estuaries, B is the putatively impacted estuary. The condition of B can be assessed by comparing all estuaries in the design. If B is impacted then A = C, but A ≠ B and C ≠ B. This increases the certainty that detected differences are due to impacts rather than inherent differences between all estuaries.

The strength of this approach is further reinforced in this study by the inclusion of an additional estuary located within a catchment with similar human impacts, say for the sake of illustration, estuary D. If both estuaries have significant human impacts, then we would once again expect that B and D differ from A and C. Estuaries B and D may also differ from one another, but overall we would expect that these estuaries would appear be more similar to each other than they are to either of the reference estuaries (A and C). We will refer to this analytical approach repeatedly when interpreting the biological, nutrient and environmental variables analysed throughout the remainder of this report.

This approach is contingent, as discussed, on the selection of estuaries that are comparable in terms of their geographical proximity, tidal range, geomorphology and size; all factors known to influence the biology, ecology and chemistry of estuaries (Edgar et al. 1999, 2000, Hirst 2004). Hirst et al. (*in review*) show that the greatest variation in these paired reference estuary designs for a common biological indicator, benthic macroinvertebrates, occurs spatially within estuaries (approx. 70-80%) compared to between estuaries (15-20%). Temporal variability by comparison accounts for a very small proportion (<10%) of the total variability. Inevitably, compromises will need to be made in the selection of appropriate reference estuaries in some cases, as few estuaries in Tasmania are completely free of human impacts.

Moreover, for unusual systems like large, drowned-river valleys (e.g. Derwent, Tamar estuaries) there may not be any corresponding reference estuaries to select from, limiting the general application of this approach.

Project outline and objectives

This report is written as an adjunct to a previous study that assessed the condition of four estuaries: the Black, Detention, Duck and Montagu estuaries in NW Tasmania in 2004/05. This report should be read in conjunction with this previous report (Hirst et al. 2005) which provides further background information on the status of the estuaries, detailed benthic habitat maps and more in depth information on sediment grain size and macroinvertebrate community composition.

The previous study compared water quality (specifically dissolved nutrient levels), sediments and invertebrate communities between four river estuaries in NW Tasmania over two seasons (spring/autumn). Two of these estuaries, the Duck and Montagu River estuaries, are located within highly impacted river catchments (as assessed independently by DPIWE, Horner et al. 2003; Pinto et al. 2003), whilst the Black and Detention River estuaries are located within relatively unmodified catchments supporting some grazing in the lower catchment, but mostly forested (Edgar et al. 1999). Historically, large tracts of native vegetation have been cleared from the Duck and Montagu river catchments and replaced by intensive dairy farming. Numerous studies have shown that nitrogen (N) and phosphorous (P) export from dairy pasture is substantially higher than for other agricultural and forestry land-uses (see review in Drewry et al. 2006) and consequently, nutrient loadings of N and P for these two river catchments are amongst the highest in Tasmania (Bobbi et al. 2003). A summary of catchment properties for each estuary are shown in Table 1.

Table 1 Summary of river catchment information displaying catchment size, human population density and % cleared of native vegetation. Also shown are AusRivAS scores and nutrient loadings for catchments where available.

	Reference		'Impacted'	
	Black	Detention	Duck	Montagu
Catchment size (km^2)[A]	324	152	339	323
Pop. density (km^{-2})[A]	1.8	6.5	25.4	6.5
% catchment cleared[A]	13.3	19.7	50.9	23
Ave. AusRivAS score[B]	1.09	0.96	0.91	0.65
Total N load (kg/ML)[C]	N/A	N/A	1.71	2.66
Total P load (kg/ML)[C]	N/A	N/A	0.53	0.80

Source: [A]Edgar et al. (1999); [B]DPIWE Water Assessment Branch (scores<0.80 indicate impairment); [C]Bobbi et al. (2003)

Hirst et al. (2005) found that while nutrient levels were generally higher throughout the Duck and Montagu estuaries (particularly phosphorous and to a lesser extent nitrates), identifiable impacts in the form of organically enriched sediments and corresponding changes to the composition and structure of invertebrate communities were confined to the upper reaches of these estuaries. By comparison, the lower reaches of the 'impacted' and reference estuaries were largely indistinguishable from one another. We attributed this finding to tidal flushing preventing accumulation of nutrients within the lower regions of these estuaries. However, the report highlighted a number of deficiencies which detracted from the strength of the overall conclusions. These included: 1) a lack of sites in the upper part of the Detention (precluding comparison with this region of the estuary); 2) no data collected from the period of maximum

freshwater river input (June-August); and 3) no information about the algal productivity, either within the water-column or the sediments, of these estuaries. The latter is an important variable when considering eutrophic responses to elevated levels of nutrients. All these deficiencies were rectified in the current study by including upper sites from the Detention estuary, sampling estuaries throughout the year including during the peak flow periods of spring and winter and measuring both water-column and sediment chlorophyll *a* levels.

The Montagu and Duck rivers receive diffuse inputs of N and P from their respective catchments (Horner et al. 2003; Pinto et al. 2003). Most of the additional inputs of N and P are derived from anthropogenic sources, primarily fertilizer but also faeces and urine from dairy cattle. Globally, 15% of N applied as fertilizer is lost via in situ denitrification and leaching into waterways, most of this exported via groundwater or surface inputs of either dissolved or particulate N (Drewry et al. 2006). Losses of N and P are also generally linked to rainfall events, with the highest inputs recorded in the wettest seasons and years (Drewry et al. 2006). Invariably, peak loads of nitrate (NO_x) occur during winter when winter rains flush stored nitrate from soils within the catchment, conversely, when riverine inputs are at their lowest in summer, biological activity is at its highest, potentially limiting the availability of N and P.

The extent to which elevated nutrient loads impact on the ecology of estuarine ecosystems is directly dependent on the residence time of the nutrients within the estuary, and the way in which biological systems respond to, and process, nutrients. Nutrient residence time is a function of tidal flushing, freshwater input/flows and the rate at which flows are diluted by tidal waters (i.e. degree of stratification). High tidal flushing and dilution lead to low residence times, limiting the biological availability of nutrients. Conversely, low tidal flushing, coupled with high freshwater inputs contribute to the accumulation of nutrients within the estuary stimulating primary production and raising concerns about eutrophication and nuisance algal blooms.

In general, bottom sediments exceed the water column as sites for processing N and P in estuaries (Nedwell et al. 1999). Organic matter is readily degraded in the sediments to its mineralised components (primarily nitrates) through microbial oxidation. In most estuaries sediments are sinks for nitrate, sources of ammonium with relatively little flux of P between the sediment and the water-column. These nutrient fluxes are aided by the physical mixing and respiratory activities of benthic invertebrates which transport nitrate and oxygen into the sediments. Benthic algal biofilms (microphytobenthos or MPBs) also play an important role in processing nutrients in sediments by scavenging N and P at the surface reducing the flux of ammonium and phosphorous to the water column, and through the production of oxygen during photosynthesis extending the depth of the oxic layer influencing chemical processes (e.g. nitrification, denitrification) within the sediments. Benthic primary producers (primarily MPBs) also account for a high proportion of total primary production in estuaries and act as significant N sinks (Nedwell et al. 1999).

The importance of sediments in processing nutrients highlights the need to examine sediment parameters in addition to water-column properties when assessing the health of estuaries. In this study we collected information on sediment chlorophyll *a* and organic carbon content; sediment redox potential (i.e. oxidative state) and infaunal macroinvertebrate community structure. Sediment chlorophyll *a* measures the productivity of MPB within the sediment, sediment organic carbon measures the levels of organic material in the sediments whereas the redox state of the sediment provides a relative measure of the oxidative state of the sediments

(i.e. +Eh oxidizing environment high O_2; -Eh reducing environment, low O_2). Macroinvertebrates are widely utilised indicators of human impacts in coastal systems because these animals live in close association with the bottom, where organic pollutants and chemical contaminants tend to accumulate and where low-oxygen conditions are typically most severe. Benthic macroinvertebrates are sensitive to a range of human perturbations (e.g. organic and chemical pollution, siltation, dredging and changes in salinity regimes) and changes in invertebrate community composition can be directly linked to ecosystem function as these animals play a critical role in detrital decomposition, nutrient cycling (see above) and energy transfer to higher trophic levels (e.g. fish, birds).

Although the Duck and Montagu catchments deliver high nutrient loads to their respective estuaries, the previous study (Hirst et al. 2005) only detected impacts in the upper reaches of these estuaries. We attributed this effect to tidal flushing, particularly in the lower reaches. As discussed above high tidal flushing reduces nutrient residence time, limiting assimilation and primary productivity. It is therefore reasonable to conclude that a large proportion of the nutrients delivered to these estuaries are flushed directly into the ocean and that this effect is greater in the lower than in the upper reaches of these estuaries.

One indirect means of measuring the relative contribution of anthropogenic sources of nutrients, in particular nitrogen, to the productivity of these estuaries is through the use of Stable Isotopes. Stable Isotopes are powerful tools for linking nitrogen in estuarine ecosystems to land-derived sources. The basis of this approach is that different sources of nitrogen have unique 'signatures' (i.e. isotopic ratio of heavier to lighter element) whose pathway can be traced through the component parts of a system providing an integrated record of sources of N (Peterson 1999). Anthropogenic sources of nitrogen (typically run-off from fertilizer or sewage inputs) have elevated signatures that can be used to trace the fate of human impacts in aquatic ecosystems. These methods have been used elsewhere to determine the influence of anthropogenic inputs at a range of scales from localised (e.g. sewage inputs; Constanzo et al. 2003, Piola et al. 2006) to broader scales differentiating catchments and estuaries (Moore and Suthers 2005, Martinetto et al. 2006). In this study we compared signatures of N of sediments, pacific oysters, burrowing crabs and infaunal amphipods for estuaries with high and low anthropogenic inputs. Unfortunately, there are no Stable Isotopes for P limiting this approach to N. Further details are provided in the methods section.

Project aims

The main aims of this study were to:

1. Provide a comprehensive assessment of the condition of the putatively impacted estuaries (Duck and Montagu Rivers) relative to the reference estuaries (Black and Detention Rivers) using the analytical framework outlined above,
2. Evaluate the efficacy of indicator variables (sediment and water-column based) using this analytical framework. We considered which variables were robust and potentially reliable measures of estuarine condition with respect to temporal (between seasons) and spatial (within estuaries) consistency; and accordingly, make recommendations regarding the use of indicator variables for future assessment/monitoring programs.
3. Develop methods for quantifying nutrient impacts associated with agricultural nutrient inputs using Stable Isotope techniques. This will allow us to directly assess the link between anthropogenic inputs and the observed biological changes, rather than simply inferring such links, and

4. Further increase our knowledge of the ecology of estuarine ecosystems in north-west Tasmania, previously neglected ecosystems.

Methods

Sampling design

The main analytical focus of this study was to compare estuaries using the 'reference-impacted' design outlined in the introduction. This was undertaken to assess the condition of two putatively impacted estuaries – the Duck and Montagu estuaries – relative to two adjacent estuaries deemed to be relatively free of human impacts – the Black and Detention estuaries (hereafter reference estuaries). In addition, comparisons between estuaries were made over time (October 2005 – July 2006) to assess the influence of time, particularly whether differences between estuaries were contingent on the time of sampling? This is important because freshwater inputs into these estuaries are highly seasonal influencing a range of ecological processes. Estuaries were also divided into clearly definable upper and lower regions identified during the previous study (Hirst et al. 2005). As with time, this was undertaken to assess whether location within estuaries influenced the outcome of comparisons between estuaries.

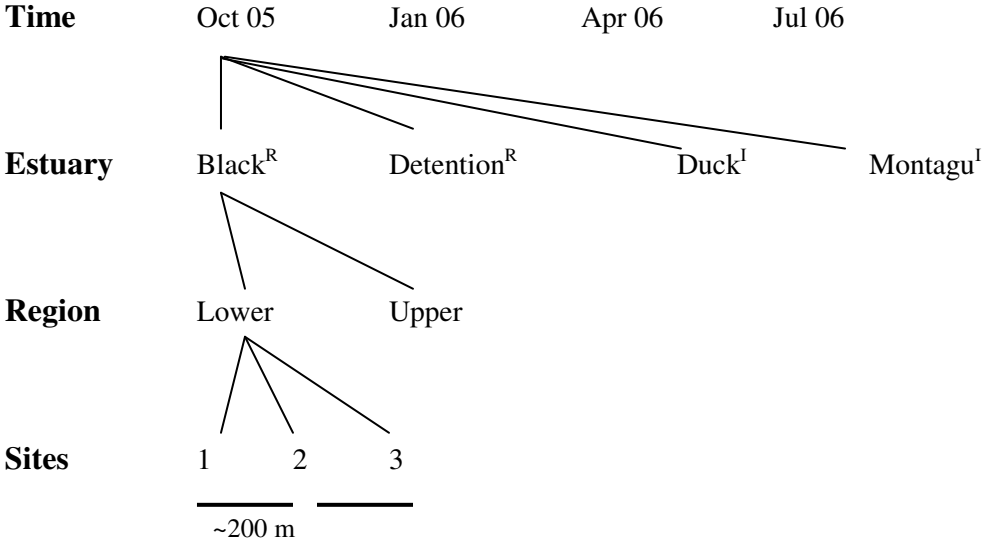

Figure 2. Diagram of sampling design diving each estuary into two regions with three sites within each region sampled on four occasions October 2005–July 2006. R and I scripts denote reference and putatively impacted status within the design.

The position and arrangement of sites within regions are shown superimposed onto maps of each of the estuaries in figure 3. GPS co-ordinates for each site are listed in appendix 1. Identical sites were visited upon each visit. Each estuary was visited on four occasions during the course of this study to measure a range of water quality (salinity, dissolved oxygen, turbidty, nutrients, chlorophyll *a*), benthic (sediment organic carbon, redox and chlorophyll *a*) and biological (macroinvertebrates) parameters. Estuaries were initially visited in October 2005 (spring) followed by visits in January (summer), April (autumn) and July (winter). These dates broadly correspond with seasonal patterns in river flows in NW Tasmania (see Fig. 4) and therefore contrast estuaries during periods of high (spring/winter) and low (summer/autumn) river inputs.

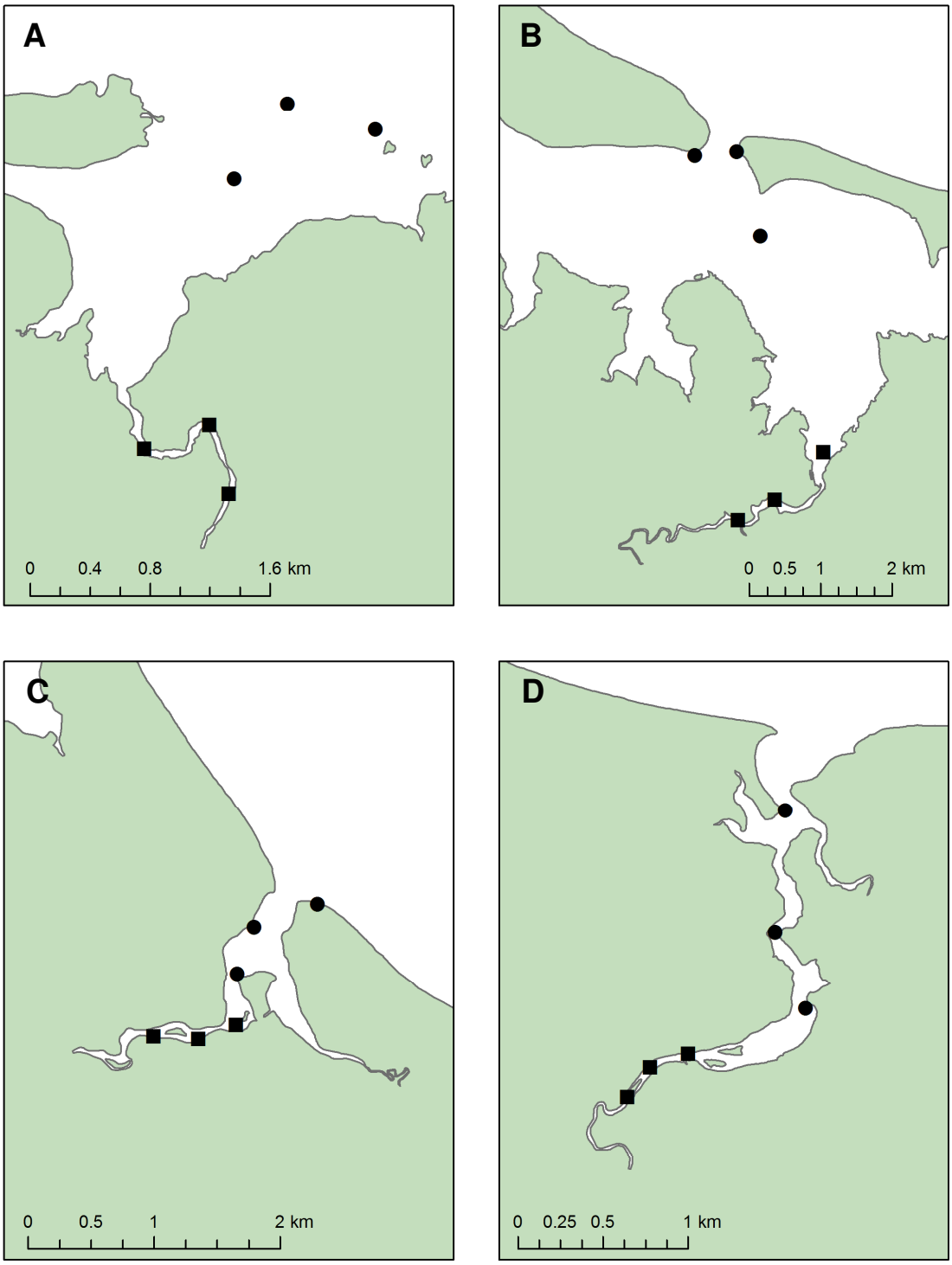

Figure 3. Location of sites within a) Montagu, b) Duck Bay, c) Black and d) Detention river estuaries. Symbols: ● lower, ■ upper sites

Sampling methods

The methods, and subsequently the results, sections of this report are divided into three main areas (water-column, sediment and macroinvertebrate assemblage parameters) with each section examining different aspects of the physicochemical and biological status of the four target estuaries.

Nutrient dynamics and physicochemical status of the water column

With the exception of salinity and dissolved oxygen all water column parameters were measured at low tide. This enabled standardization of measurements in relation to tidal phase, whilst measuring nutrient levels at their maximum concentrations (see Hirst et al. 2005).

Variables measured included (NB: all measurements were made in surface waters at LT unless otherwise stated):

1. Salinity (surface and bottom measurements) at low and high tide
2. Dissolved oxygen concentration (surface and bottom measurements) at low and high tide
3. Turbidity
4. Temperature
5. Dissolved nutrient concentrations including ammonium (NH_4-N), nitrate and nitrite (NO_x-N) and soluble reactive phosphorous (P)
6. Chlorophyll *a* concentrations

Measurements were made mid-channel (i.e. equidistant from either bank where possible) within surface waters <30 cm depth. Salinity, DO and turbidity were measured in the field using meters. Nutrient levels were determined later in the lab from water samples collected in the field. Water samples were stored on ice in the field then later frozen. Soluble ammonium, nitrate, nitrate and reactive phosphorous were analysed by Analytical Services Tasmania. Chlorophyll a levels were determined by filtering water samples in the field onto GF/F Whatman filters, chlorophyll extracted from filters with 90% v/v acetone (90% v/v) over a 24 hour period in darkness at 4 °C, then determined spectrophotometrically. Chlorophyll *a* levels were used as a proxy for water-borne microalgal biomass in this study.

Sediments

Sediment organic carbon, redox potential and chlorophyll *a* levels were determined for sediments adjacent to the water column sampling sites. Samples were taken just below the low water mark at low tide. Sediment organic carbon content was derived from 45 mm diameter cores to a depth of 50 mm; sediment chlorophyll a from three replicate micro-cores 22 mm diameter inserted to a depth of 15 mm (following Light and Beardall 1999). Sediments were frozen in the field and returned to the laboratory for analysis. Organic carbon content of sediments was determined via chemical titration. This has been found to be a more accurate method than the loss on ignition (LOI) method used previously in Hirst et al. (2005). Chlorophyll *a* was measured as a proxy for microalgal biomass in the sediments. Chlorophyll was extracted from the sediments with acetone (90% v/v) over a 24 hour period in darkness at 4 °C, and then determined spectrophotometrically. Sediment from which chlorophyll a was extracted was dried at 80 °C for 48 h and weighed allowing for chlorophyll a concentration to

be expressed as μg of chlorophyll a per g of dried sediment (ug g^{-1}). The redox potential of the sediment was measured in situ using a pH/redox probe at a depth of 30 mm below the surface. Redox potential was corrected for differences in ambient temperature between sampling dates prior to analysis.

Benthic macroinvertebrates

The diversity and composition of the soft sediment macroinvertebrate fauna in each estuary was determined using replicated sediment cores collected at each site. At each site five sediment cores (diameter = 150 mm, depth = 100 mm) were collected from subtidal sediments using a transect spanning from the low water mark to depth of approximately 0.7 m as a guide. Cores were collected at 0.0, 0.1, 0.2, 0.5 and 0.7 m depths. Cores were then sieved through a 1.0 mm-mesh sieve in the field and the portion retained was fixed in 5% buffered formalin. Salinity and DO measurements corresponding with the scale of the macroinvertebrate sampling were also made by resting the probes on the sediment. This information was later used to examine correlations between macroinvertebrate assemblage structure and environmental variables.

Samples were sorted in the lab to the lowest possible taxonomic level and the number of individuals of each taxa recorded. This information was used to calculated species richness (i.e. no. of taxa), total faunal abundance and species composition for each site by amalgamating the replicate samples (i.e. totals not means).

Data analysis

The main analytical focus of this study was to compare estuaries located within impacted catchments (Duck and Montagu) with estuaries located within less modified catchments (Black and Detention). In addition estuaries were contrasted across four times that broadly corresponded with seasonal changes in freshwater river input and across two locations within each estuary (upper and lower regions). Thus, the analytical framework for this study comprises three factors of interest: time (n = 4 levels), estuary (n = 4) and region (n = 2); with three replicate sites located within each region (see Fig. 2).

Univariate variables

Differences between estuaries, sampling dates and regions were analysed using a three-way factorial design using analysis of variance (ANOVA). Estuary and region were treated as fixed factors (i.e. represent specific comparisons between estuaries and regions), whereas time is considered a random factor in this design - sampling dates chosen randomly from a larger possible range of sampling dates. The advantage of this design is that while definitive comparisons can be made between estuaries and regions, detected changes in time can extrapolated beyond the current range of sampling dates meaning that seasonal and other temporal patterns can be inferred. This alters the hypothesis tests used to calculate the F-ratio and hence the P-value in the analysis. Differences between estuaries (where detected) were identified using *post-hoc* Tukeys HSD tests contrasting individual estuaries. This test corrects for Type I error (i.e. finding a statistically significant effect as result of chance). Prior to analysis, data were transformed (usually naural log or acrsine√ mathematical transformations) where necessary to comply with the assumptions of ANOVA (i.e. data is normally distributed).

Macroinvertebrate community composition (multivariate data)

Similarity-based multivariate statistical techniques provide a more powerful means of measuring change in invertebrate structure across estuaries because data for each individual species is retained in the analysis. A full description of these methods is available in Clarke and Warwick (2001). The Bray-Curtis index of similarity – the standard similarity measure used for ecological data due to its ability to cope with large number of zeros that typify community-level data – was used to measure similarity between sites. The index measures similarity between sites in terms of the composition of species (species shared) and their relative frequencies. Log(x+1) data transformations were applied in this instance to reduce the overall influence of very abundant species in the analysis, rather than to meet the assumptions of any statistical tests. Such transformations are standard practise in multivariate statistical analysis.

Similarity between sites was represented in two-dimensions using non-metric multidimensional scaling (nMDS) ordination (PRIMER v 6.0 software package). The position of estuaries and regions was superimposed onto nMDS ordination plots to aid interpretation (see Results). However, it is important to remember that ordination is not a statistical test, but simply a means of graphically representing the sample similarity relationships inherent in the underlying triangular similarity matrix. Valid statistical comparison of groups instead requires the incorporation of a null hypothesis (i.e. no difference in community composition between groups) similar to those utilised in ANOVA tests. Here we use the multivariate equivalent of ANOVA, permutational analysis of variance (PERMANOVA; Anderson 2005), to test for differences in assemblage composition between time, estuaries and regions using the three-way factorial design. The statistical significance of each term was tested using a randomisation test (n = 999 permutations).

Correlations between invertebrate community structure and abiotic variables were examined using the BIOENV routine in PRIMER 6. BIOENV computes the Spearman rank correlation between the ranked distances between pairs of sites inherent in the similarity matrix and the abiotic data. The significance of the test statistic (rho) was tested using a randomisation test (n = 999 permutations).

Stable Isotope analysis

To determine the extent of coupling between estuarine food-web components in April and October (corresponding with low/high freshwater inputs) and anthropogenic sources of nitrogen we measured N and C stable isotope ratios in the tissues of three species common to all estuaries and sediments. These species included the burrowing crab *Macrophthalamus latifrons,* the infaunal amphipod *Paracorophium* sp. (both restricted to the upper reaches) and wild pacific oysters (*Crassostrea gigas*) largely restricted to the lower reaches of these estuaries. We also measured N stable isotope ratios for benthic sediments (i.e. sediment organic matter) collected from each of the estuaries. Field samples for each of these target groups were collected following the layout shown in table 2.

Table 2. Stable isotope sample design used in this study displaying the number of samples collected in the lower and upper reaches of each estuary in April and October 2006

	Lower	Upper
M. latifrons (crabs)		3 sites (n = 3 crabs site^{-1})
Paracorophium sp. (amphipods)**		3 sites*
C. gigas (oysters)	3 sites (n = 3 oysters site^{-1})	
Sediments	3 sites*	3 sites*

* replicate samples amalgamated for sample analysis
** sufficient numbers for analysis only available in Oct. 2006

Crabs and amphipods were collected from three sites in the upper reaches, oysters at three sites in the lower reaches and sediments at three sites in the upper and lower reaches corresponding with the sampling sites used in the above design (see Fig. 3). Crabs and oysters were collected by hand. Amphipods were collected using replicate cores which were subsequently amalgamated to provide sufficient numbers for analysis. Sediments were collected using five 22 mm diameter micro-cores inserted to a depth of 15 mm and subsequently amalgamated for later analysis. All samples were frozen prior to analysis. Crabs and amphipods were left in seawater for 24 hours to allow gut evacuation prior to freezing.

In the laboratory samples were de-frosted and rinsed with deionised water (except sediments). We used tissue extracted from the chelipeds (pincers) and legs of crabs following Guest et al. (2004) and oyster adductor muscle following the recommendations of Piola et al. (2006). Amphipod samples comprised >100 individuals combined. Samples were dried at 60 °C and ground to a fine powder. Isotope analyses of nitrogen and carbon were performed by the Stable Isotope Facility in the Research School of Biological Sciences, Australian National University using a mass spectrometer. Isotope analyses of nitrogen in sediments were performed by CSIRO Marine and Atmospheric Research, Hobart. The ratios of $^{15}N/^{14}N$ (i.e. $\delta^{15}N$) and $^{13}C/^{12}C$ (i.e. $\delta^{13}C$) are expressed as the relative difference in parts per thousand ( ) between the sample and a recognised international standard (atmospheric nitrogen for N and Vienna Pee Dee Belemnite limestone carbonate for C).

Stable isotope ratios of N and C for crabs, oysters, amphipods and sediments were compared between estuaries, seasons and regions (where applicable) using ANOVA. All terms were treated as fixed factors. Differences between estuaries (where detected) were determined using *post-hoc* Tukeys HSD tests contrasting individual estuaries.

Results

River flows

Rivers in the NW display distinctly seasonal patterns in river discharge, corresponding with rainfall patterns in the region. Peak flows occur during winter/spring (June – October), whilst reduced flows occur over the summer months (January – May) (Gurung and Dayaratne 2003).

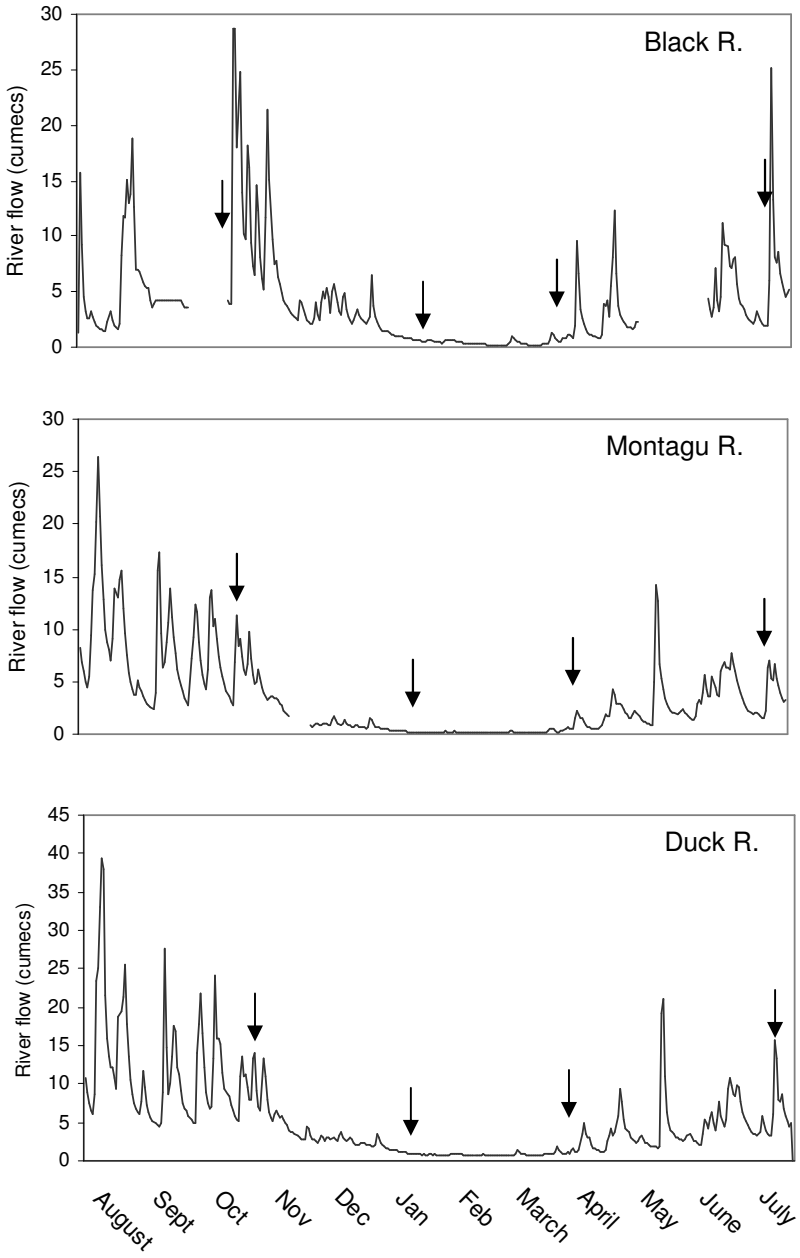

Figure 4. Mean daily discharge (m^3 sec^{-1}) for the Black, Montagu and Duck Rivers for the period August 2005 – July 2006. Spring (Oct. 05), summer (Jan. 06), autumn (April 06) and winter (July 06) sampling events are indicated on the plots. Source: Tasmanian DPIW

River flows for three of the four estuarine catchments are shown for the period August 2005–July 2006 in figure 4 (i.e. Black, Montagu and Duck rivers). There is no gauging station for the fourth catchment, the smallest of the four, the Detention River and flow patterns for this estuary are assumed from the other three, adjacent, catchments. The wetter months are characterised by peaks in discharge (floods) indicative of greater flow variability, whereas the summer months experience universally lower flows.

Rainfall in 2006 has been uncharacteristically low by regional standards (Tasmanian Bureau of Meteorology 2006 summary). In fact in many parts of north-west Tasmania annual rainfalls for 2006 have been the lowest on record. Consequently, winter river flows have been low in comparison to the long-term average. Figure 5 shows the mean monthly flows for the previous year to August 2006 and the 30-year mean for the same period. These plots indicate that winter flows in June-August 2006 were up to 50% lower than the 30-yr mean, whereas spring flows in the preceding year (2005) appear to be higher than the mean.

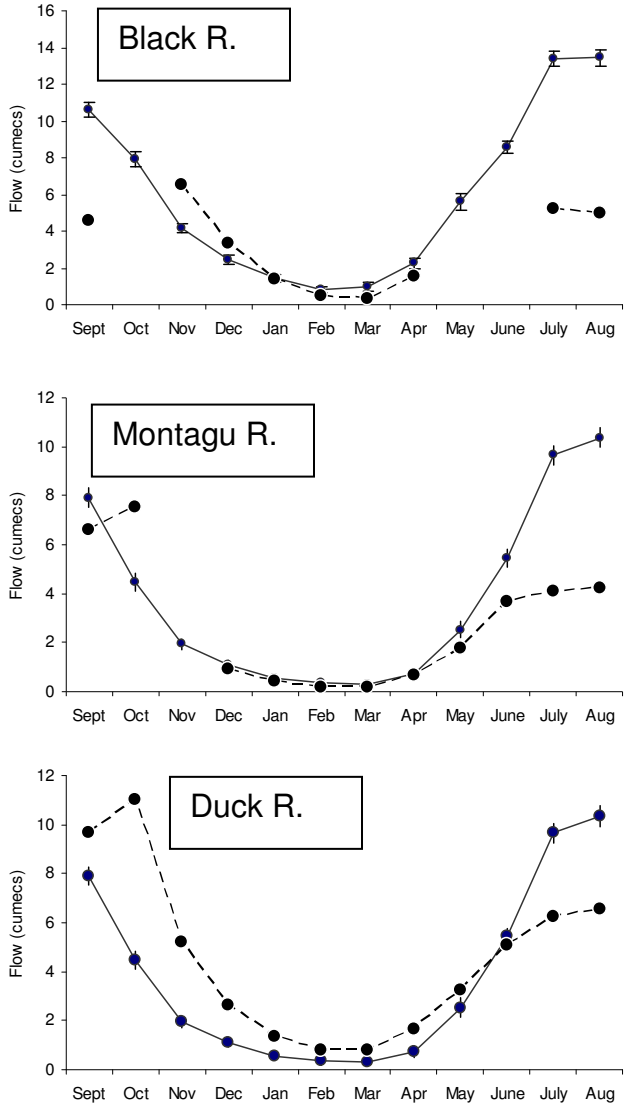

Figure 5. Mean daily river discharge per month for the Black, Montagu and Duck rivers. Lines = 30-yr mean (±SE) (–●–) and 2005-06 (--●--) flows. Source: Tasmanian DPIW

Sediments

Sediments in the estuaries were dominated by fine sands (particles within the 0.125–0.25 mm range), to the extent that the majority of sites sampled comprised >70% fine sands by weight (Hirst et al. 2005). Nevertheless, sites located in the upper and lower regions of the estuaries can be clearly distinguished on the basis of the silt/clay (<0.63 mm particles) content of the sediments, although the pattern is less clear for the Black estuary (Fig. 6). Sites in the upper regions of the Duck, Montagu and Detention estuaries contained a greater percentage of silt/clay particles. Lower sites in the Duck and Montagu estuaries had a lower percentage of silt/clay particles, than the corresponding lower sites in the Black and Detention estuaries.

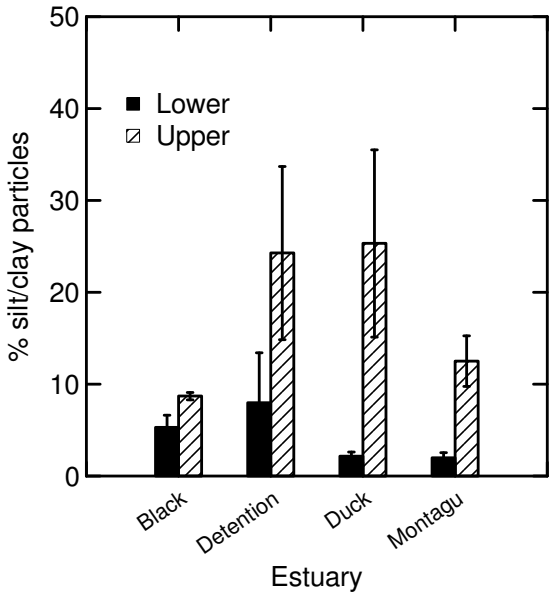

Figure 6. Mean percent silt/clay content (±SE) of sites sampled in the upper and lower regions of each of the estuaries. Source: Hirst et al. (2005)

Higher silt/clay content (and the associated organic material) is mirrored by the darker-muddier appearance of the benthos in the upper compared to the lower regions; and indeed this was one of the criteria used to delineate upper and lower sites within the sampling design. The change from lighter-sandier sediments to darker-muddier sediments is also clearly visible in aerial photos of estuaries and can be viewed in habitat maps of the estuaries shown in Hirst et al. (2005) figures 33-36.

Temperature

Predictably surface water temperature followed a seasonal pattern throughout the course of this study. The highest water temperatures were recorded during January, whilst the lowest temperatures were recorded in July (Fig. 7).

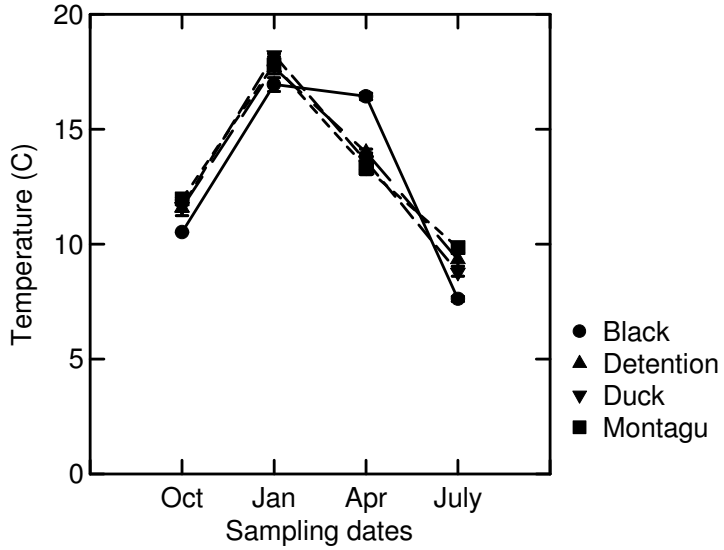

Figure 7. Seasonal changes in water temperature averaged across sites within estuaries.

Physicochemical and dissolved nutrient concentrations

Salinity

In an effort to detect the presence of a halocline over changing tides four salinity measures were collected at each site: surface and bottom salinity at high and low tide. In general there was a halocline in all estuaries, although the disparity between surface and bottom salinities varied between sampling times, tides and position with the estuary (Fig. 8). In general haloclines (i.e. difference between surface – bottom salinity) were greater at low tide compared to high tides, within the upper compared to lower reaches and during October (spring) and July (winter). Surface salinities displayed a clear seasonal pattern over time with lower salinities recorded in July and October compared to April (Table 3, Fig. 8). As expected, salinities were lower in the upper compared to the lower reaches, although this pattern is clearer for the Duck and Montagu than the Black and Detention estuaries (hence the significant interaction between the estuary and region (E*R)).

Table 3. Three-way ANOVA testing for differences in surface salinity @ LT between sampling times, estuaries and regions. Also shown are the outcome and direction of post-hoc statistical tests comparing estuaries and regions. ns: non-significant

Source	df	MS	F	P	Post-hoc test
Time (T)	3	1356.74	63.58	<0.001	
Estuary (E)	3	42.55	0.63	ns	
Region (R)	1	4398.33	247.31	0.001	lower>upper
T*E	9	67.43	3.16	0.003	
T*R	3	17.79	0.83	ns	
E*R	3	583.34	55.20	<0.001	
T*E*R	9	10.57	0.50	ns	
Residual	64	21.34			

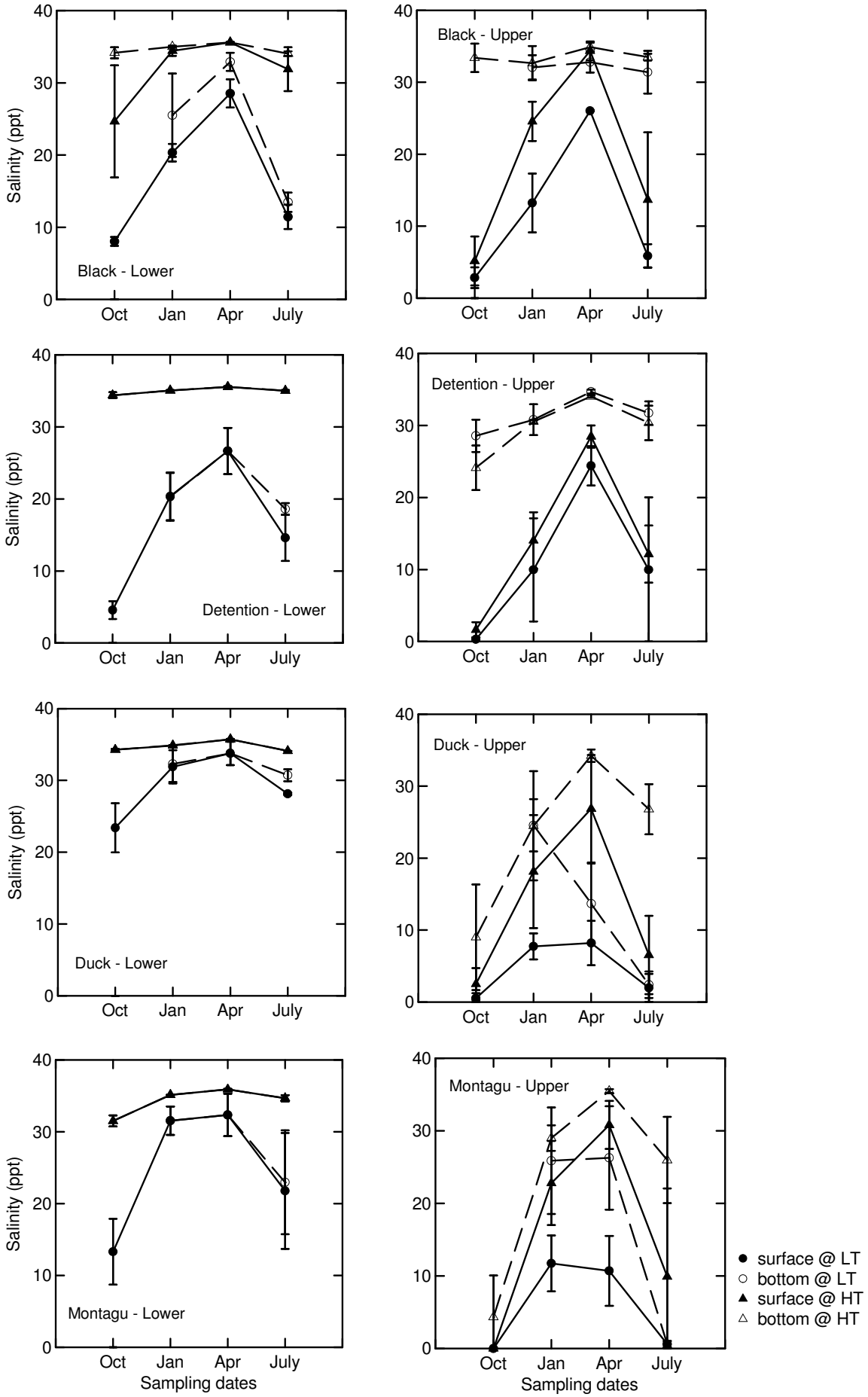

Figure 8. Mean salinity (±SE) in the lower and upper regions of each estuary Oct. 05 – July 06.

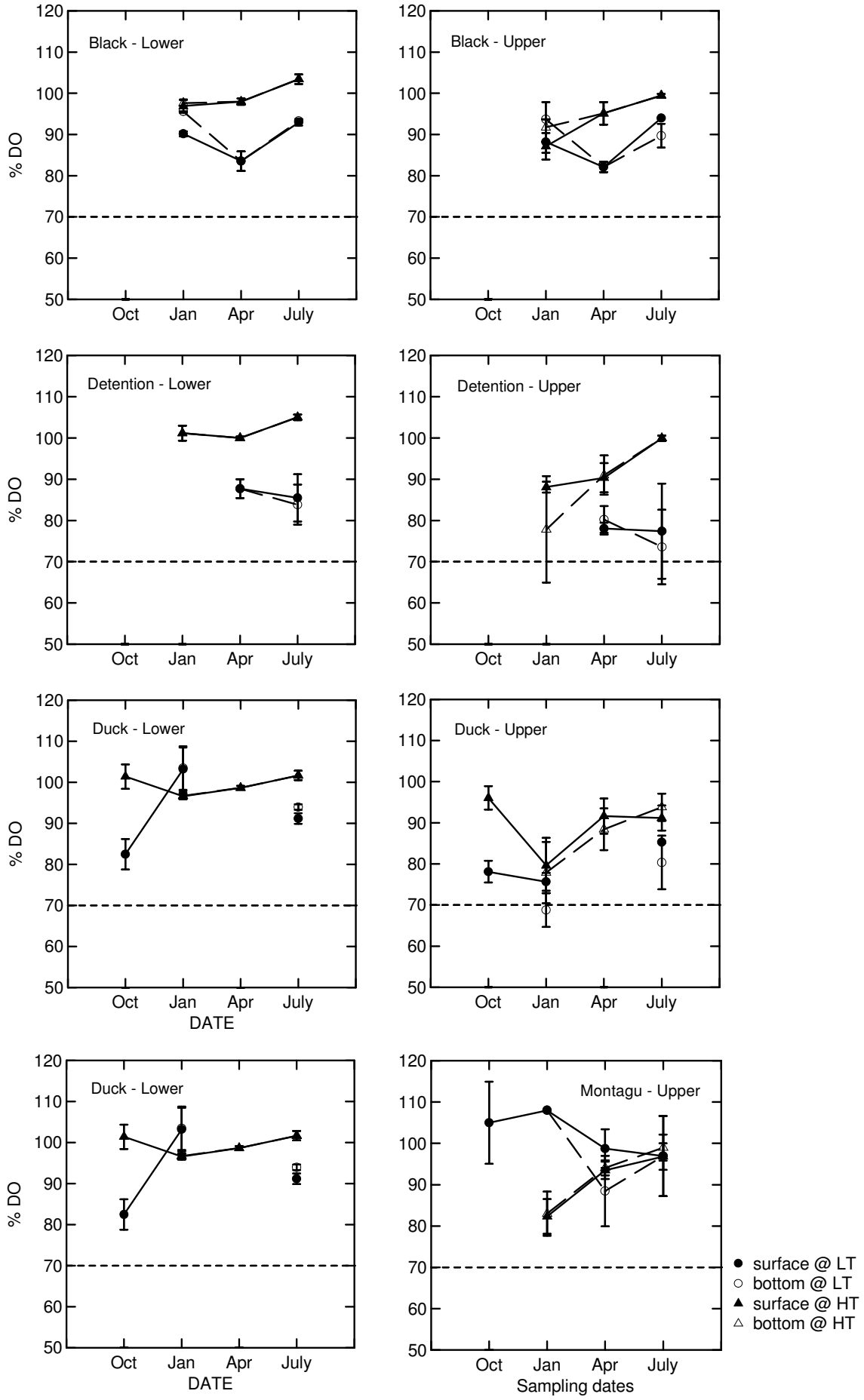

Figure 9. Mean dissolved oxygen % saturation (±SE) in lower and upper regions Oct. 05 – July 06.

Dissolved oxygen concentrations

Dissolved oxygen concentrations (DO) for surface and bottom waters at low and high tide are shown for the upper and lower reaches of each estuary in figure 9. There were a number of data gaps (e.g. October data for Black and Detention estuaries), consequently, no effort has been made to analyse the DO data. The plots reveal no clear trends in DO concentrations. Generally, DO concentrations were high suggesting that waters were well oxygenated within these estuaries. The upper reaches of these estuaries were less well oxygenated than the lower reaches, but still in excess of values considered to be a problem for aquatic life (i.e. the 70% cut-off shown on the plots).

Turbidity

Turbidity levels were significantly higher in the Duck and Montagu estuaries, but only in the upper region of these estuaries (as indicated by the strong statistical interaction between the estuary and region factors) (Table 4; Fig. 10). Estuary differences were also inconsistent over time (i.e. significant time x estuary interaction term). This is likely to be due to overall reductions in turbidity, particularly in the Duck and Montagu estuaries, during the summer (Jan and April) compared to the winter months.

Table 4. Three-way ANOVA testing for differences in log transformed turbidity levels between sampling times, estuaries and regions. Also shown are the outcome and direction of post-hoc statistical tests comparing estuaries and regions. ns: non-significant

Source	df	MS	F	P	Post-hoc test
Time (T)	3	0.081	5.400	0.002	
Estuary (E)	3	0.886	17.373	<0.001	Du>M>Bl,Dn
Region (R)	1	1.813	151.083	0.003	Upper>Lower
T*E	9	0.051	3.400	0.002	
T*R	3	0.012	0.800	ns	
E*R	3	0.345	17.250	<0.001	
T*E*R	9	0.02	1.333	ns	
Residual	63	0.015			

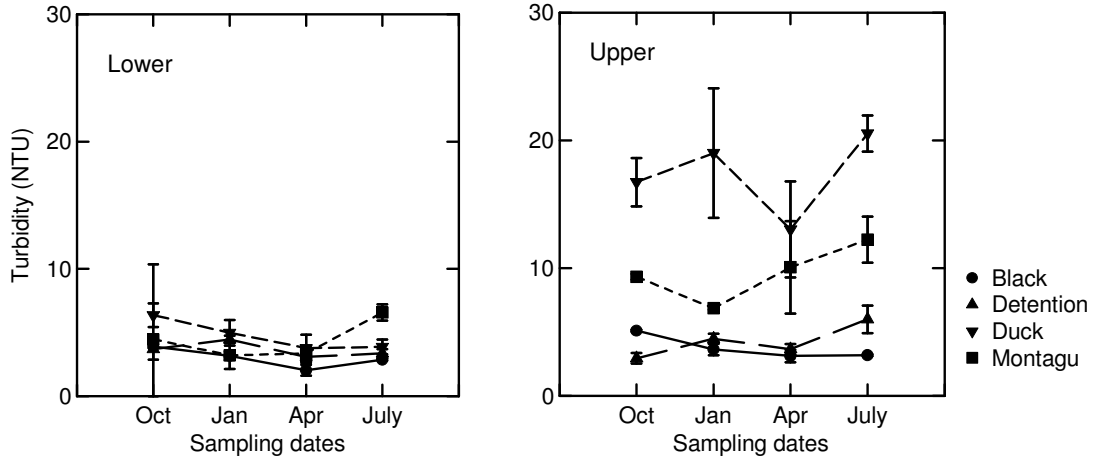

Figure 10. Mean turbidity (±SE) levels in the lower and upper regions of estuaries Oct. 05 – July 06.

Ammonium concentrations

The ammonium form of dissolved nitrogen in these systems did not significantly differ between estuaries or regions (Table 5; see Fig. 11). However, concentrations of ammonium did vary significantly between sampling times - relaying a seasonal pattern whereby higher concentrations of ammonium were measured in October and July compared to January and April (Fig. 11).

Table 5. Three-way ANOVA testing for differences in log transformed NH_4-N concentrations between sampling times, estuaries and regions. ns: non-significant

Source	df	MS	F	P
Time (T)	3	1.956	37.615	<0.001
Estuary (E)	3	4.04	3.091	ns
Region (R)	1	0.37	0.794	ns
T*E	9	1.307	25.135	<0.001
T*R	3	0.466	8.962	<0.001
E*R	3	1.075	10.047	0.008
T*E*R	9	0.107	2.058	0.047
Residual	62	0.052		

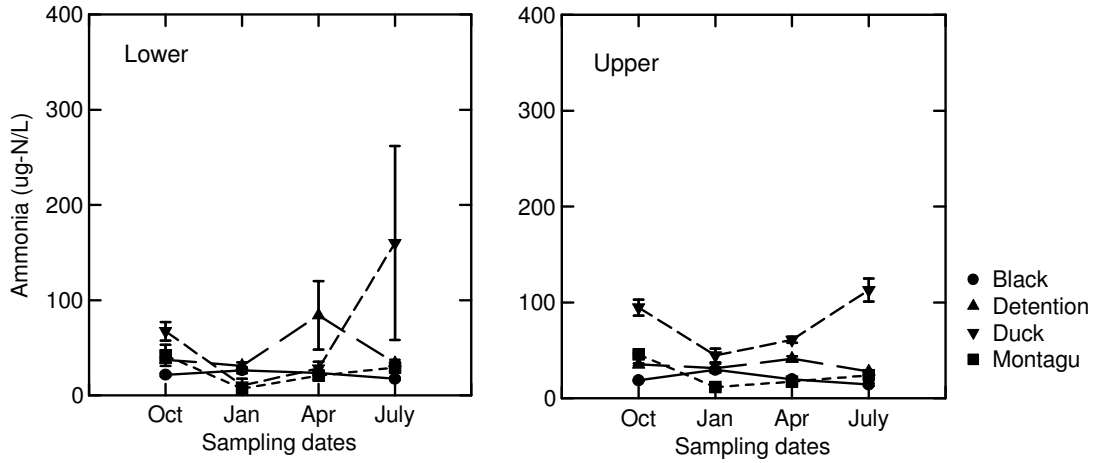

Figure 11. Ammonium concentrations (±SE) in the lower and upper regions of each estuary Oct.05 – July 06.

Nitrate/nitrite concentrations

Dissolved NOx levels displayed a clear temporal (seasonal) pattern (see Fig. 12). In general NOx concentrations were higher in October and July and this pattern was evident across both the upper and lower regions of the estuaries sampled. The strength of this seasonal pattern also varied between estuaries (i.e. as illustrated by significant time x estuary interaction) and was more pronounced for the Montagu compared to the Black estuary (see Fig. 12). NOx levels did not significantly vary between estuaries (table 6).

Table 6. Three-way ANOVA testing for differences in log transformed NO_x-N concentrations between sampling times, estuaries and regions. ns: non-significant

Source	df	MS	F	P
Time (T)	3	15.225	61.391	<0.001
Estuary (E)	3	4.686	3.828	ns
Region (R)	1	19.055	19.504	0.04
T*E	9	1.224	4.935	<0.001
T*R	3	0.977	3.940	0.012
E*R	3	3.021	8.035	0.015
T*E*R	9	0.376	1.516	ns
Residual	62	0.248		

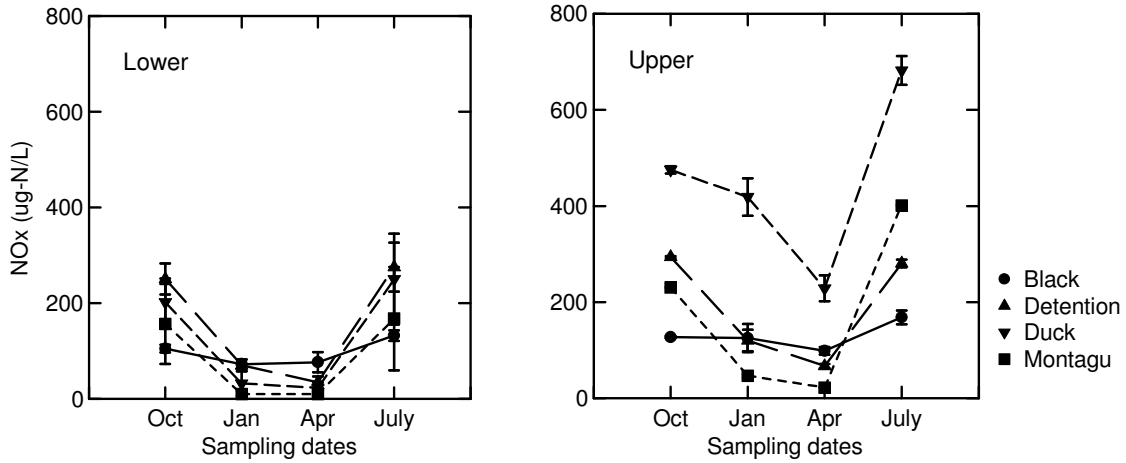

Figure 12. Nitrate/nitrite (NOx) concentrations (±SE) in the lower and upper regions of each estuary Oct. 05 – July 06.

Dissolved reactive phosphorous (DRP)

DRP levels differed significantly between estuaries and sampling times, but not between regions (Table 7). As with the NOx data there was a clear seasonal pattern in DRP concentrations, particularly evident for the Duck and Montagu estuaries (Fig. 13). Concentrations of DRP were highest in July and October and lowest during April and the end of summer. The pattern was similar for both the upper and lower regions. Post-hoc statistical tests indicated that DRP concentrations were highest in the Duck estuary, followed by the Montagu, Detention and Black estuaries. The tests indicated that DPR concentrations in each of the estuaries were statistically significant from one another (Table 7). However, the strong statistical interaction between time x estuary ($P<0.001$) indicates that estuary-to-estuary differences varied over time. This suggests that statistical differences between estuaries may only be detected at specific times of the year, for example, when DRP levels are at their highest during winter and spring.

Table 7. Three-way ANOVA testing for differences in log transformed dissolved phosophorous concentrations between sampling times, estuaries and regions. Also shown are the outcome and direction of post-hoc statistical tests comparing estuaries. ns: non-significant

Source	df	MS	F	P	Post-hoc test
Time (T)	3	5.759	45.346	<0.001	
Estuary (E)	3	21.251	35.596	<0.001	Du>M>Dn>Bl
Region (R)	1	0.015	0.142	ns	
T*E	9	0.597	4.701	<0.001	
T*R	3	0.106	0.835	ns	
E*R	3	0.934	10.860	0.004	
T*E*R	9	0.086	0.677	ns	
Residual	62	0.127			

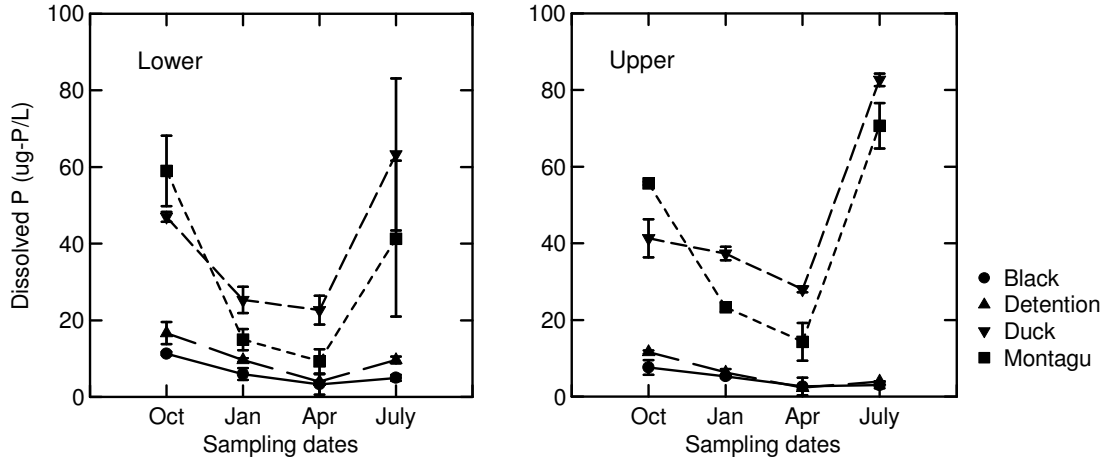

Figure 13. Dissolved reactive phosphorous concentrations (±SE) in the lower and upper regions of each estuary Oct. 05 – July 06.

Chlorophyll *a* concentrations

Chlorophyll *a* concentrations varied significantly through time and between estuaries, but not between regions (Table 8). Chlorophyll *a* displayed a seasonal pattern broadly corresponding with changes in water temperature (see Fig. 7). The highest chlorophyll *a* concentrations were recorded in spring (Oct 05) and summer (Jan 06), whilst the lowest concentrations were recorded in winter (July 06) (Fig. 14). This pattern was consistent between regions, although is clearer amongst the upper sites. Overall, chlorophyll *a* levels appear higher for the upper region, but were not significantly different from lower sites in this study (Table 8). This is largely due to high variability in chlorophyll *a* measurement recorded at the site level – as indicated by the high standard error bars plotted on the graphs (see Fig 14).

Table 8. Three-way ANOVA testing for differences in log transformed Chlorophyll *a* concentrations between sampling times, estuaries and regions. Also shown are the outcome and direction of post-hoc statistical tests comparing estuaries. ns: non-significant

Source	df	MS	F	P	Post-hoc test
Time (T)	3	0.268	12.762	<0.001	
Estuary (E)	3	0.38	7.755	0.01	Du>Dn,Bl; M>Bl
Region (R)	1	0.501	14.735	ns	
T*E	9	0.049	2.333	ns	
T*R	3	0.034	1.619	ns	
E*R	3	0.037	1.542	ns	
T*E*R	9	0.024	1.143	ns	
Residual	63	0.021			

Overall chlorophyll *a* levels were highest in the Duck estuary, followed by the Montagu, Detention and Black estuaries. Post-hoc statistical tests indicated that concentrations in the Duck were significantly higher than the Black and Detention estuaries, whereas concentrations in the Montagu were significantly higher than those in the Black estuary, but could not be distinguished from concentrations in the Detention estuary (Table 8).

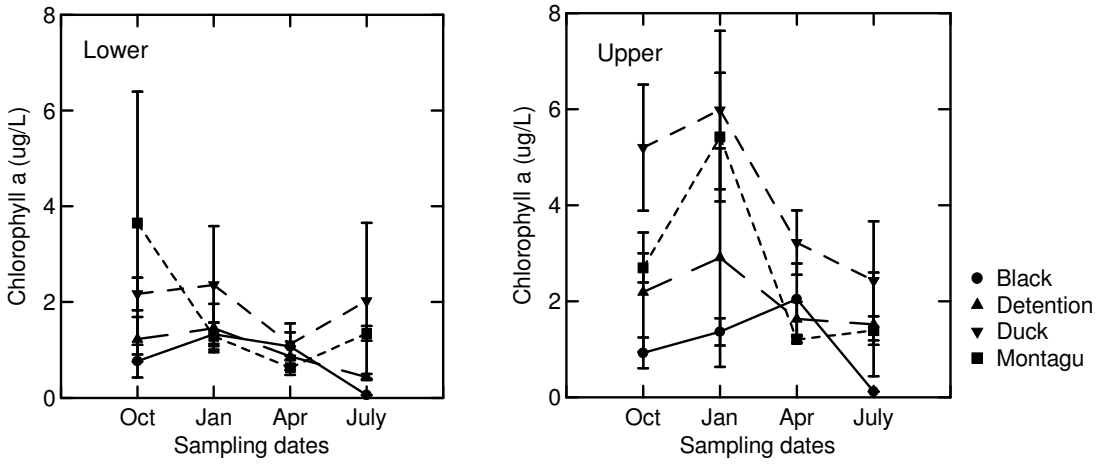

Figure 14. Chlorophyll *a* concentrations (±SE) in the lower and upper regions of each estuary Oct. 05 – July 06.

Benthic variables

Sediment organic carbon content

Percent organic carbon content within the sediments varied substantially between regions within estuaries, but not between estuaries or sampling times (Table 9; Fig. 15). Sediments in the upper regions contained a much greater proportion of organic carbon than the sites sampled within the lower region (which were uniformly low). This pattern was closely associated with differences in the sediments found between the upper and lower regions. Sites from the lower regions invariably comprised sandy, coarser sediments, whereas the upper sites comprised muddy/silty sediments containing a greater abundance of fine organic material (commensurate with the depositional nature of the upper/riverine reaches of these estuaries). This pattern was consistent across estuaries and sampling times (i.e. absence of any significant statistical interactions between region and estuary/time).

Table 9. Three-way ANOVA testing for differences in arsine √ transformed % sediment organic carbon between sampling times, estuaries and regions. ns: non-significant

Source	df	MS	F	P
Time (T)	3	0.004	2.0	ns
Estuary (E)	3	0.002	2.0	ns
Region (R)	1	0.284	284	<0.001
T*E	9	0.001	0.5	ns
T*R	3	0.001	0.5	ns
E*R	3	0.003	3.0	ns
T*E*R	9	0.001	0.5	ns
Residual	64	0.002		

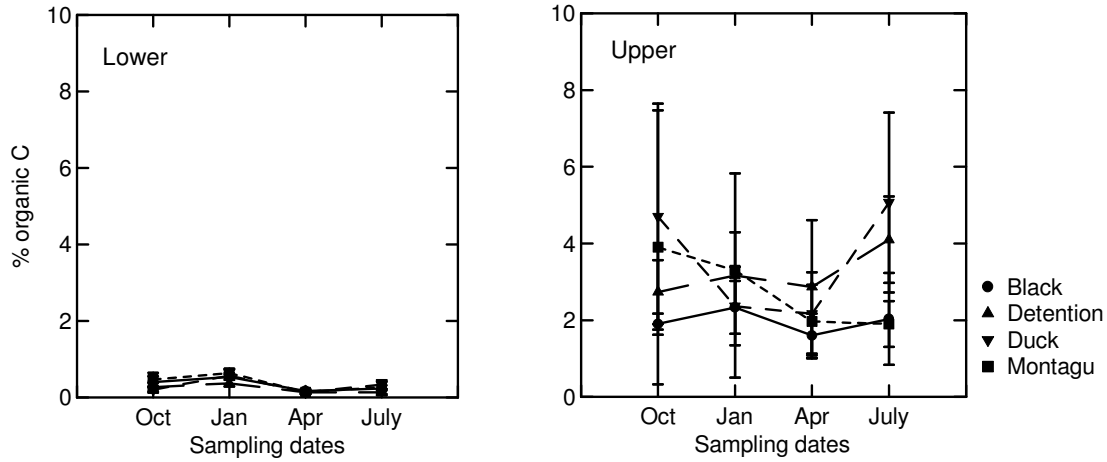

Figure 15. Percent sediment organic carbon (±SE) in lower and upper regions of estuaries Oct. 05 – July 06.

Sediment redox potential

Due to the high variability of redox measurements no differences were detected between sampling times, estuaries or regions (ANOVA table not shown). Although, redox measurements made in the upper regions were consistently more negative, redox recorded in the lower regions were by contrast, with the exception of the Black estuary, highly variable, preventing any clear comparison of regions (Fig. 16). Many measurements in the lower regions of the Detention, Duck and Montagu estuaries were as negative as those recorded from the upper regions.

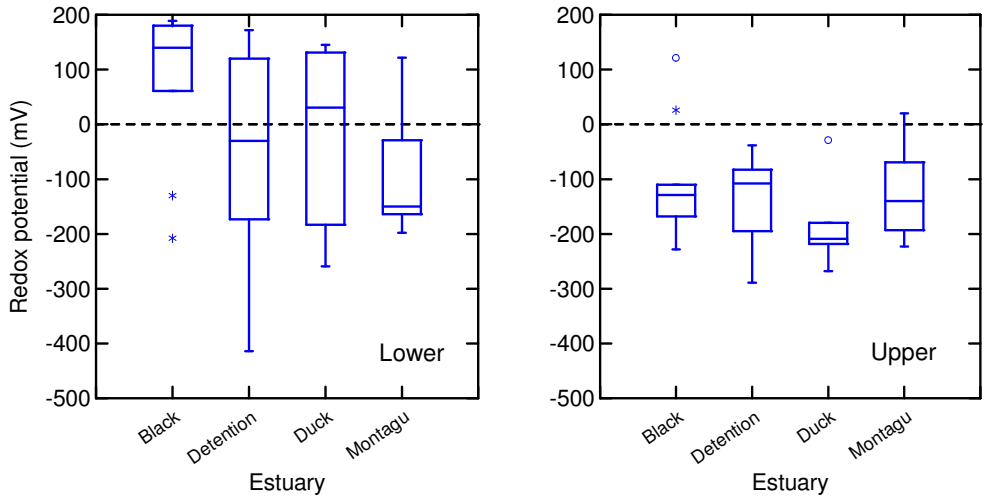

Figure 16. Boxplots showing the range of redox values recorded across estuaries for lower and upper regions (pooled across sampling dates). Values in the positive range indicate greater oxidative potential (oxic sediments). Negative values indicate greater reductive potential, indicative of low oxygen conditions. Horizontal bars are medians, boxes are interquartiles and vertical bars are ranges excluding outliers (*) and far outliers (°).

Sediment chlorophyll *a*

We used the mean of three replicate measurements taken at each site to run the analyses shown below. This is because MPB biomass is typically very patchy at small spatial scales (Underwood and Kromkamp 1999). Ninety percent of sites had coefficients of variation (CV = (standard deviation of the 3 replicate measurements/the mean) x 100) less than 50% (i.e. standard deviation is less 50% of the mean); and 40% of sites had CVs <10%. Moreover, while CVs varied between sampling dates, estuaries and regions none of these factors were significant (when analysed using 3-way ANOVA), indicating no inherent bias in terms of variability amongst these factors. In other words replicates collected in one month, estuary or region were no more variable than any other month, estuary or region. However, sites with CVs > 50% should be treated with the caution during analysis.

Sediment chlorophyll a levels varied significantly between estuaries and regions, but not sampling times (Table 10; Fig. 17). Sediment chla levels were generally much higher in the upper compared to the lower region, although a significant statistical interaction between estuary and region indicated this pattern may not be consistent across estuaries. The other pattern of note relates to differences between estuaries. Post-hoc statistical tests indicated that Chla levels were higher in the Duck and Montagu estuaries, albeit largely amongst the upper sites.

Table 10. Three-way ANOVA testing for differences in sediment Chlorophyll a concentrations between sampling times, estuaries and regions. Also shown are the outcome and direction of post-hoc tests comparing estuaries and regions. ns: non-significant

Source	df	MS	F	P	Post-hoc test
Time (T)	3	7.487	0.356	ns	
Estuary (E)	3	185.445	4.983	0.05	Du, Mn>Bl, Dn
Region (R)	1	1090.593	105.026	0.003	Upper>Lower
T*E	9	37.216	1.768	ns	
T*R	3	10.384	0.493	ns	

E*R	3	77.962	9.615	0.008
T*E*R	9	8.108	0.385	ns
Residual	64	21.053		

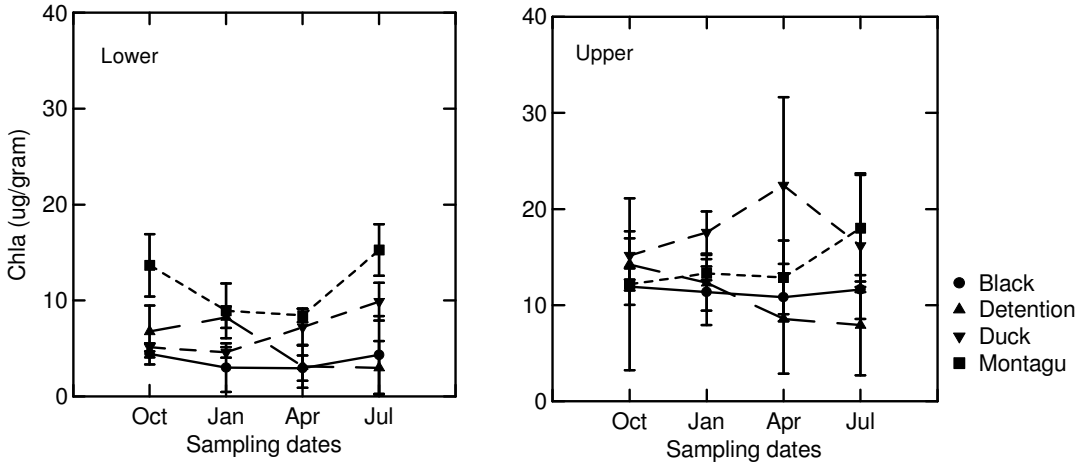

Figure 17. Mean (±SE) sediment chlorophyll a concentrations (µg Chla g^{-1} dry weight sediment) between estuaries and sampling dates for sites in the lower and upper regions.

Macroinvertebrate assemblages

A total of 81 taxa were collected from the four estuaries surveyed in this study. This included 30 crustacean, 24 polychaete and 20 mollusc taxa. Overall more taxa were collected from the lower than the upper regions, although the disparity was greater for the Duck and Montagu estuaries (Table 11). This is possibly because the lower reaches of these estuaries are larger and contain a greater range of soft-sediment habitats. The most abundant species collected were *Paracorophium* sp. (amphipod), *Arthritica semen* (bivalve), *Paphies* sp. (bivalve), *Hydrococcus brazieri* (gastropod) and *Nephtys australiensis* (polychaete) (total n > 1000 individuals). A list of taxa collected during this study is given in appendix 2.

Table 11. Total number of species collected in the upper and lower regions partitioned by each estuary.

Estuary	Region	No. species
Black	Upper	31
	Lower	37
Detention	Upper	27
	Lower	34
Duck	Upper	29
	Lower	48
Montagu	Upper	26
	Lower	50
All estuaries	Upper	44
	Lower	76
Survey Total		81

Univarate analyses

Species richness varied significantly between estuaries and regions, but not times (Table 12). In general species richness was higher in the lower reaches (Fig. 18), although a statistical interaction between estuary and region indicates that the extent of regional differences in species richness varied between estuaries (e.g. see Detention in summer and winter). Post-hoc statistical tests between estuaries showed samples collected from the Montagu had higher species richness than the other three estuaries.

Table 12. Three-way ANOVA testing for differences in species richness between sampling times, estuaries and regions. Also shown are the outcome and direction of post-hoc tests comparing estuaries and regions.

Source	df	MS	F	P	Post-hoc test
Time (T)	3	17.844	1.564	ns	
Estuary (E)	3	60.483	8.959	0.009	M>Du, Dn, Bl
Region (R)	1	231.26	62.894	0.008	Lower>Upper
T*E	9	6.751	0.592	ns	
T*R	3	3.677	0.322	ns	
E*R	3	83.26	6.626	<0.001	
T*E*R	9	12.566	1.102	ns	
Residual	64	11.406			

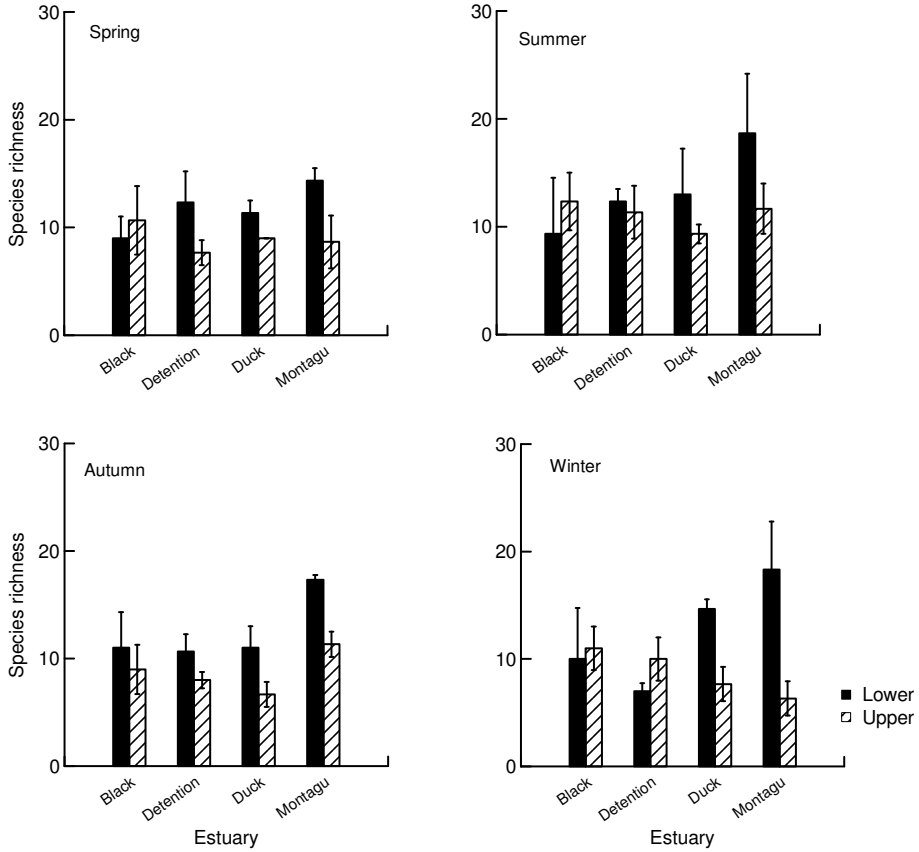

Figure 18. Mean (± SE) species richness per site for estuaries and regions by sampling times.

Macroinvertebrate densities varied significantly between estuaries, but not times and regions (Table 13). In general densities were greater in the upper reaches of the estuaries with the exception of autumn (in part explaining the time x region interaction) and when presumably *Paracorophium* sp. densities were lowest at the end of summer. Post-hoc statistical tests indicated that faunal densities in the Montagu and Detention estuaries were higher than the Black and Duck, although these are difficult to discern in figure 19.

Table 13. Three-way ANOVA testing for differences in faunal abundance between sampling times, estuaries and regions. Also shown are the outcome and direction of post-hoc tests comparing estuaries and regions.

Source	df	MS	F	P	Post-hoc test
Time (T)	3	1.584	2.234	ns	
Estuary (E)	3	4.502	5.398	0.03	M,Dn>Du,Bl
Region (R)	1	23.165	7.863	ns	
T*E	9	0.834	1.176	ns	
T*R	3	2.946	4.155	0.009	
E*R	3	0.971	1.258	ns	
T*E*R	9	0.772	1.089	ns	
Residual	64	0.709			

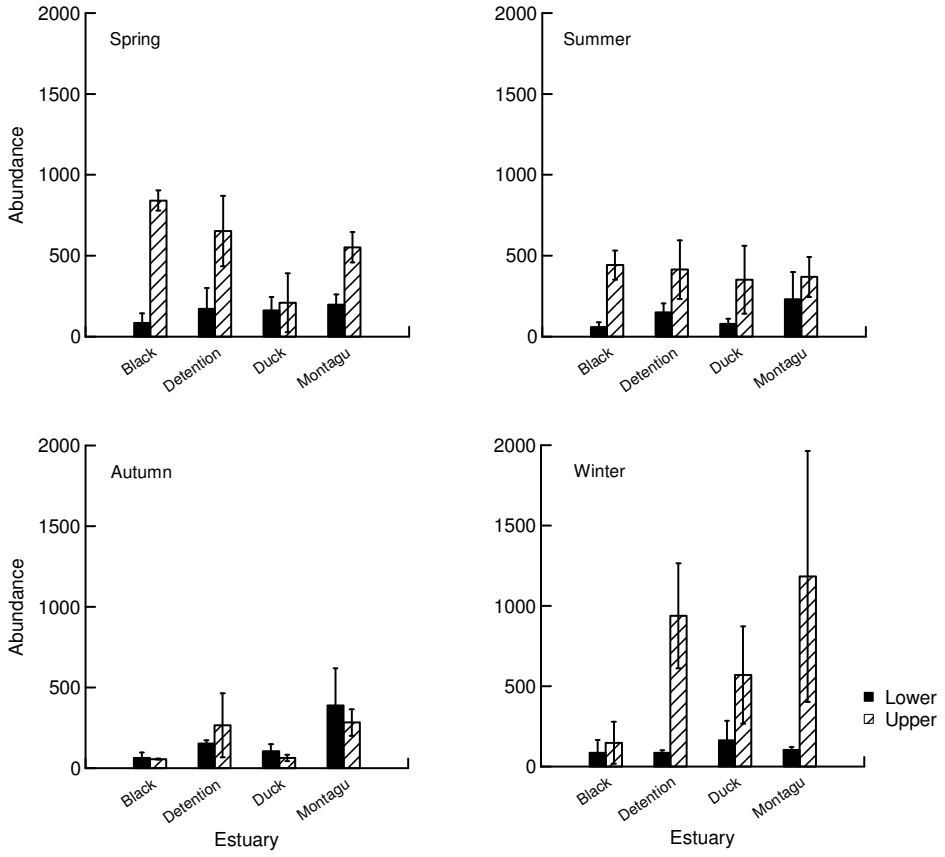

Figure 19. Mean (± SE) abundance per site for estuaries and regions by sampling times.

Multivariate community analyses

Permutational multivariate analysis of variance (PERMANOVA) was used to test for differences in macroinvertebrate community structure between sampling times, estuaries and regions within estuaries. PERMANOVA found significant differences between seasonal sampling dates, estuaries and regions, however the majority of variance in assemblage structure (as demonstrated by the proportional variance shown in Table 14) was expressed at the within-estuary scale: between regions (upper – lower contrast) and individual sites (i.e. residual variance).

Table 14. Three-way permutational multivariate analysis of variance (PERMANOVA) based on Bray-Curtis distance matrix of log(x + 1) transformed macrofaunal assemblage data. Also shown is the pseudo-multivariate estimated variance for each term and their constituent interactions. Estimated variance components expressed as a proportion of the residual variance for each term in the model.

Source	df	MS	F	P	Prop. variance
Time (T)	3	3692.6	2.998	<0.001	0.08
Estuary (E)	3	8366.4	6.793	<0.001	0.24
Region (R)	1	71113.7	25.274	0.007	1.18
T*E	9	1293.8	1.051	ns	0.01
T*R	3	2813.6	2.285	0.008	0.11
E*R	3	6414.4	5.208	<0.001	0.35
T*E*R	9	1203.5	0.977	ns	0.00
Residual	64	1231.6			1.00
Total	95				

Significant differences were also evident between estuaries, although the amount of variance explained is substantially less. Post-hoc contrasts between individual estuaries indicate that this is because assemblages in the Montagu estuaries differed significantly from those in the other three estuaries (Table 15). The lack of significant differences between the other three estuaries (i.e Duck, Detention and Black estuaries) indicates that macroinvertebrate assemblages in these three estuaries were largely indistinguishable.

Table 15. Post-hoc tests comparing estuaries (PERMANOVA analysis of multivariate data)

Contrast	t-statistic	P
Bl vs Dn	1.66	0.025
Bl vs Du	1.67	0.019
Bl vs M	2.32	0.003*
Dn vs Du	1.61	0.036
Dn vs M	2.14	0.008*
Du vs M	1.96	0.008*

*sig. following Bonferroni correction for multiple tests, $a = 0.008$

Importantly, the absence of a significant interaction between estuary and time indicates that comparisons between estuaries remained consistent regardless of when the estuaries were sampled. Moreover, seasonal differences accounted for only a small proportion of the total variance, suggesting temporal shifts in assemblage structure are small in comparison to spatial variation within- and between-estuaries. Overall the greatest differences in assemblage structure were found between sites located in the upper and lower regions (Table 14; Fig. 20).

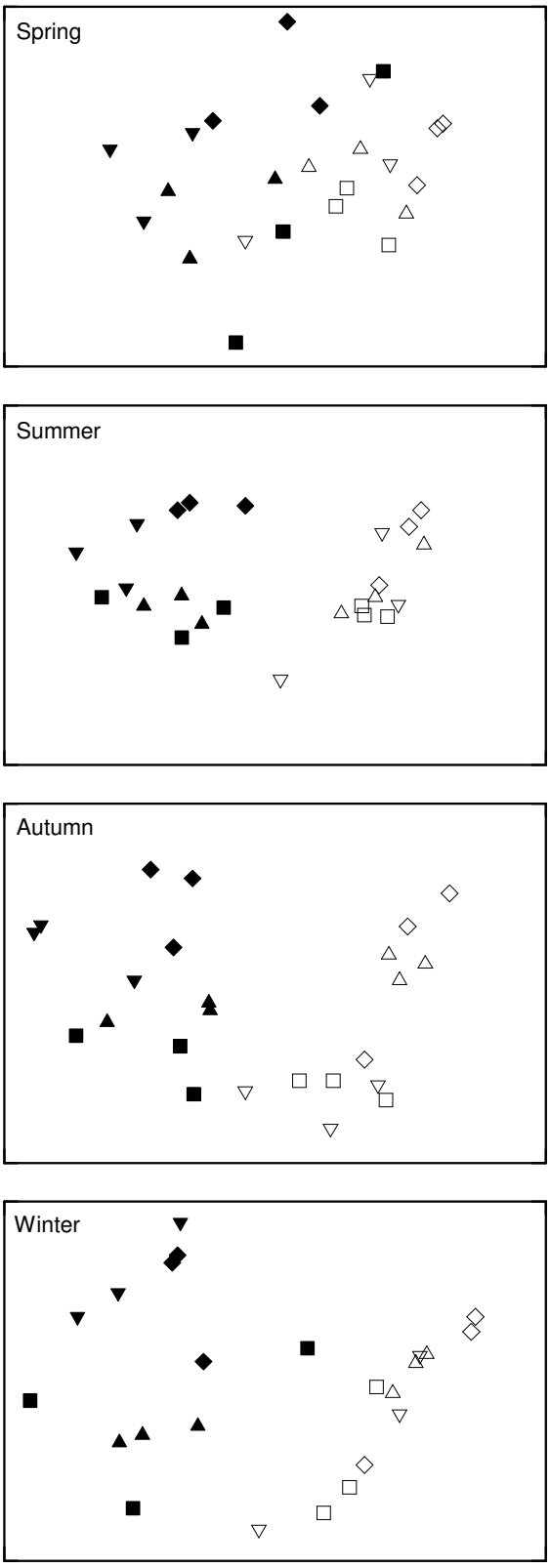

Fig. 20. nMDS ordination of macroinvertebrate assemblage displaying the relative position of sites within estuaries in two-dimensions for each season: spring (Oct), summer (Jan), autumn (Apr) and winter (July) (closed symbols: lower; open symbols: upper; ◇ Montagu; □ Black; △ Detention; ▽ Duck). Stress = 0.16

Upper and lower sites can be clearly distinguished from one another in the nMDS ordination plots for summer, autumn and winter sampling events, although differences in community structure were less clear during spring (Fig. 20). Macroinvertebrate species that distinguished the upper from the lower regions of the estuaries are shown in table 16 which summarizes SIMPER (similarity percentages) analyses comparing regions. Taxa characteristic of the upper reaches were: *Paracorophium* sp., *Arthritica semen*, *Nepthys australiensis*, Chironomid larvae, *Boccardiellla limnicola* and *Simplisetia aequisetis*. *Paracorophium* sp. and *Arthritica semen* densities were high in the upper reaches of all four estuaries (see also below). Taxa characteristic of the lower reaches included: *Paphies* sp. *Mysella donaciformis*, *Urohaustorius halei* and *Hydrococcus brazieri.* Other taxa commonly encountered during this survey displayed no clear pattern in terms of distribution between the upper and lower regions (e.g. *Scoloplos normalis, Biffarius* spp.) (Table 16).

Table 16. SIMPER (similarity percentages) analysis displaying macroinvertebrate taxa with high contributions (> 2% dissim.) to Bray-Curtis dissimilarity between the upper and lower regions of the four estuaries. Taxa are ranked by % dissimilarity. Species indicative of the upper reaches of these estuaries are underlined. The 14 taxa shown collectively explain 70% of the dissimilarity between the upper and lower regions of these estuaries.

Taxa	Order	Mean abund. site^{-1}		Diss./SD	% Diss.
		Lower	Upper		
Paracorophium sp.	Amphipoda	12.4	323.4	1.50	12.80
Paphies sp.	Bivalve	51.2	2.1	1.80	10.37
Arthritica semen	Bivalve	1.6	57.3	1.76	9.09
Mysella donaciformis	Bivalve	8.1	1.8	1.22	4.83
Nephtys australiensis	Polychaete	5.9	16.1	1.18	4.80
Chironomidae spp.	Insect	1.0	18.4	0.90	4.34
Scoloplos normalis	Polychaete	2.6	3.9	1.13	3.64
Boccardiella limnicola	Polychaete	0.8	15.0	0.77	3.48
Urohaustorius halei	Amphipoda	6.6	0.0	0.81	3.42
Nassarius spp.	Gastropod	3.5	0.8	1.32	3.29
Hydrococcus brazieri	Gastropod	29.9	0.4	0.71	3.07
Simplisetia aequisetis	Polychaete	0.3	6.7	0.75	2.66
Katelysia scalarina	Bivalve	3.6	0.3	0.86	2.61
Biffarius spp.	Decapod	0.8	1.9	0.74	2.39

Distribution and abundance of common estuarine invertebrate species

The distribution and abundance of five common estuarine species: *Paracorophium* sp. (amphipod), *Boccardiella limnicola*, *Nepthys australiensis*, *Scoloplos normalis* (polychaetes) and *Arthritica semen* (bivalve) were analysed separately using 3 way-ANOVA. All species, with the exception of *Paracorophium* sp., displayed no temporal patterns in abundance. *Paracorophium* sp. on the other hand showed significant declines in abundance during summer and autumn (Jan and April) (Table 17, Fig. 21). *Paracorophium* sp. numbers appear to be strongly influenced by river flows and commensurate changes in salinity and nutrient levels, while the abundance of the other common macroinvertebrate species appear largely independent of seasonal river flows. All species, with the exception of *S. normalis*, were more abundant in the upper compared to the lower reaches of these estuaries. In the case of *Paracorophium* sp., *A. semen* and *B. limnicola* these species are largely restricted to the upper reaches of these estuaries (Figs. 21 & 22).

Table 17. Summary of ANOVA analyses indicating level of significant differences (P<0.05) in mean abundance per site between sampling times, estuaries and regions for five common estuarine species.

Species	Results of ANOVA
N. australiensis	No temporal pattern; no diff. b/w estuaries; upper>lower abundance ($F_{1,3}$=32.3; P=0.03)
A. semen	No temporal pattern; M>Du,Bl,Dn ($F_{3,9}$=9.4; P=0.008); upper>>lower abundance ($F_{1,3}$=28.3; P=0.03)
S. normalis	No pattern.
Paracorophium sp.	Seasonal pattern: spring/winter>summer/autumn ($F_{3,64}$=28.4; P<0.001); no diff. b/w estuaries; upper>>lower abundance ($F_{1,3}$=29.2; P=0.03)
B. limnicola	No temporal pattern; M>>Du,Bl,Dn ($F_{3,9}$ = 27.9; P<0.001); upper>>lower abundance ($F_{1,3}$=37.5, P=0.01)

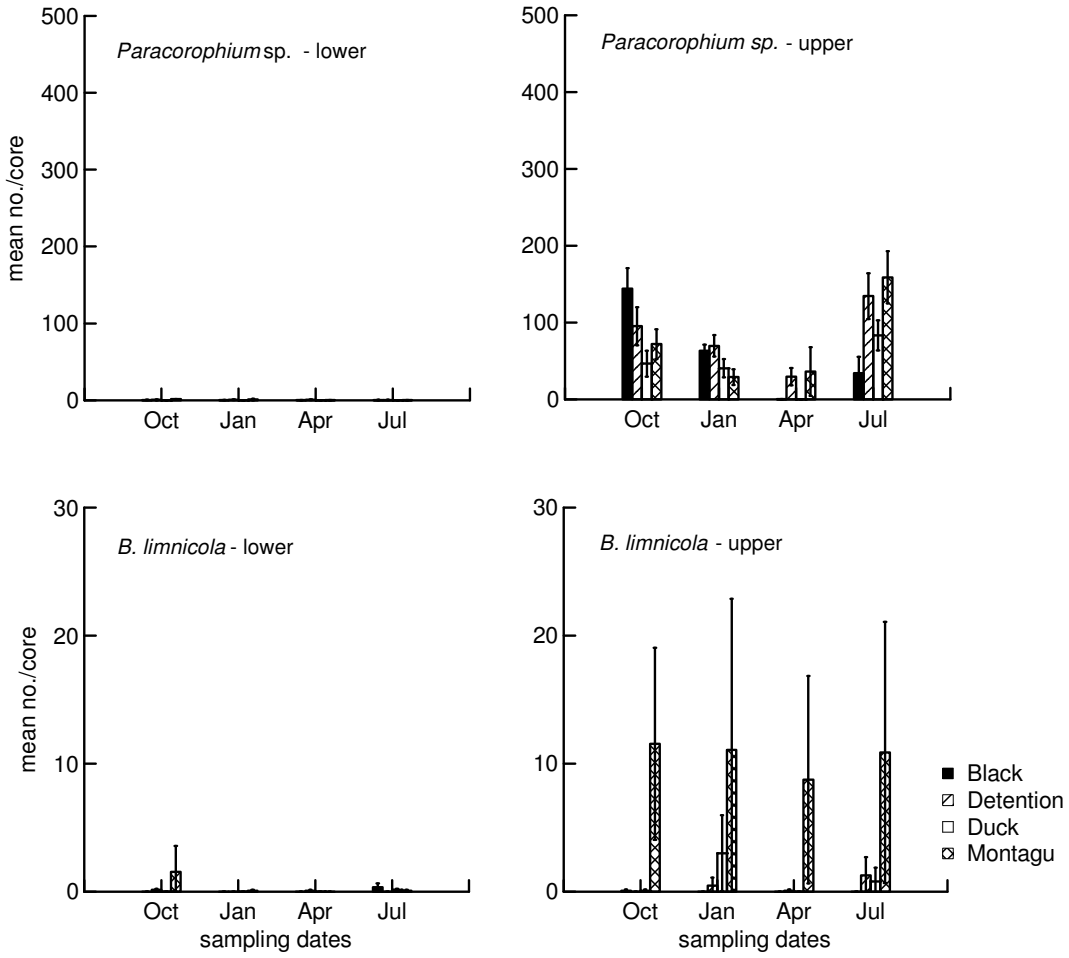

Fig. 21. Mean (±SE) abundance for *Paracorophium* sp. and *B. limnicola* displaying distribution patterns between seasons, estuaries and regions (upper and lower).

Only *A. semen* and *B. limnicola* displayed significant differences between estuaries (Table 17). Both *A. semen* and *B. limnicola* were much more abundant in the Montagu estuary (Figs 21 & 22) – mirroring the results of the multivariate analyses. Other species varied considerably between estuaries, notably *S. normalis* and *Paracorophium* sp., but no consistent pattern was detected over the course of this study.

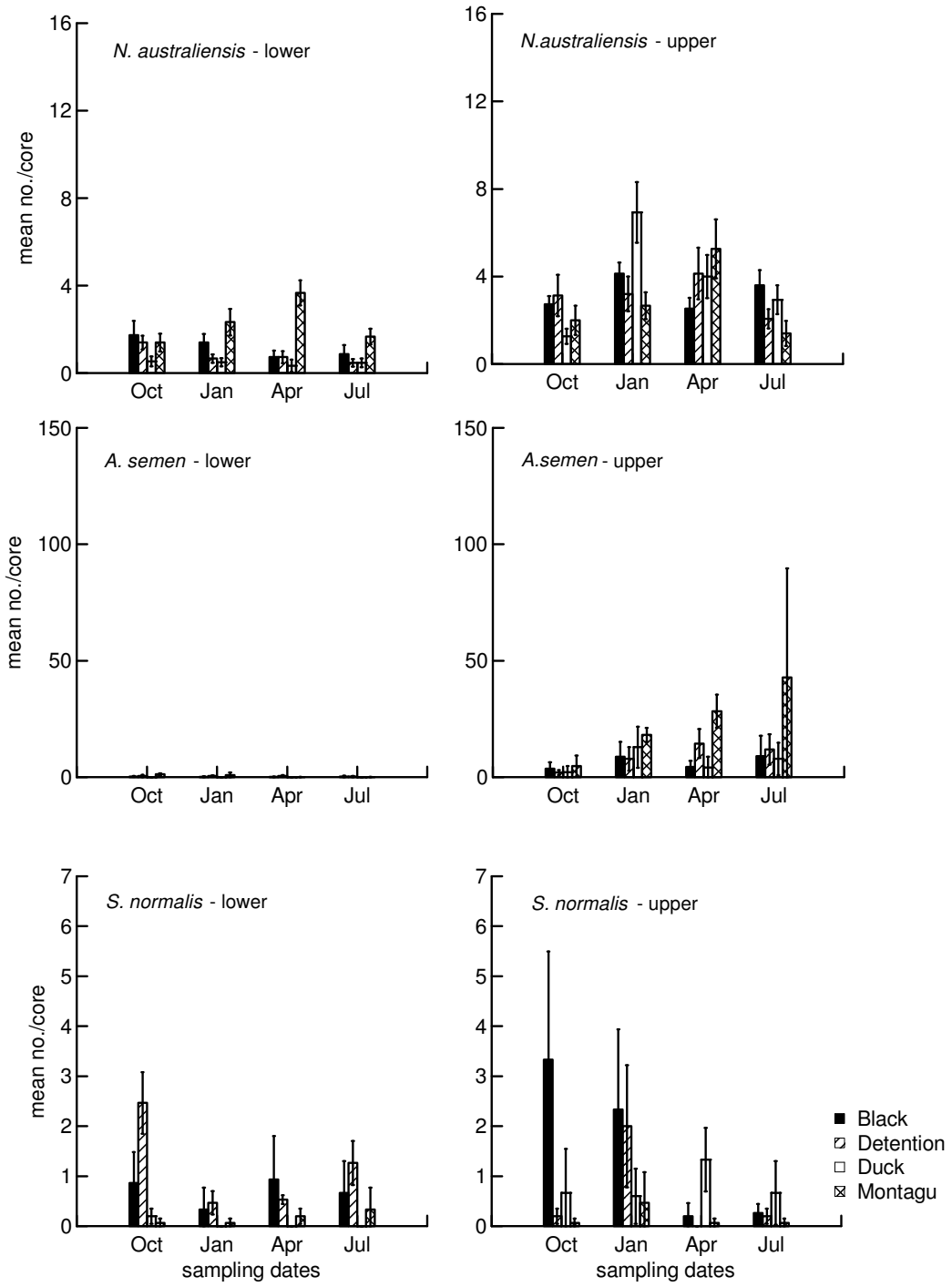

Fig. 22. Mean (±SE) abundance for *N. australiensis*, *A. semen* and *S. normalis* displaying distribution patterns between seasons, estuaries and regions (upper and lower).

Correlations between macroinvertebrates and environmental variables

The strongest correlations between macroinvertebrate assemblage structure and environmental variables were recorded for variables that described differences between the upper and lower reaches of the estuaries (Table 18). This is because location within estuary (i.e. situarted upper and lower regions) was found to be one of most important drivers of macroinvertebrate assemblage structure across estuaries and sampling dates (see table 14 and fig. 20). Consequently, variables that differed appreciably between regions were found to have significant, but not necessarily high, correlations with the structure of macroinvertebrate assemblages. These included salinity, % organic C, % silt/clay content and mean sediment Chl a levels (Table 18). This pattern can be illustrated by superimposing important explanatory variables such as salinity and % organic C onto the nMDS ordination of the macroinvertebrate assemblages surveyed (i.e. Fig. 20). Salinities are generally higher in the lower region of the estuary (Fig. 23a), whereas organic C levels were higher in the upper region and correspondingly lower in the lower region (Fig. 23b). By comparison, environmental variables that varied significantly over time, for example temperature (see Fig. 7 – temperature plot), explained very little of the variance in the structure of the macroinvertebrate assemblages in this study (ρ=0.011, P=0.304). This is because temporal changes in community assemblage structure were relatively insignificant compared to those explained by spatial elements (see PERMANOVA variance components in Table 14).

Table 18. BIOENV results displaying the Spearman rank correlation coefficient (ρ) for correlations between environmental variables with macroinvertebrate assemblage structure.

Environmental variable	Rho (ρ)	P
Salinity (surf) @ LT	0.415	<0.001
% Sediment Org C	0.305	<0.001
% silt/clay particles	0.252	<0.001
Mean sediment Chl a	0.200	<0.001
% DO (surf) @ LT	0.053	0.054
Temperature	0.011	0.304
Best combination of variables:		
Salinity, % Org C and Mean Sed Chl a	0.444	<0.001

Although significant, the low correlations (0.200-0.415) suggest that only a small component of the variance exhibited in the structure of these assemblages can be explained by these variables (i.e. approx. 10-20% of the variance). When examined together (i.e. using a multiple regression type approach) the best combination of variables – salinity, % organic C and sediment Chl a levels - provided little additional explanatory power (Table 18). This is because these variables collectively explain the same upstream environmental gradient apparent between the upper and lower reaches of these estuaries. Nonetheless, these analyses confirm that changes in water chemistry, particularly salinity, and sediment structure are important drivers of inverterbrate community structure in these estuaries. In particular estuarine communities sampled from the upper reaches of these estuaries (see section above) are associated with variable and often lower salinities and organically enriched and finer sediments (see Fig. 23).

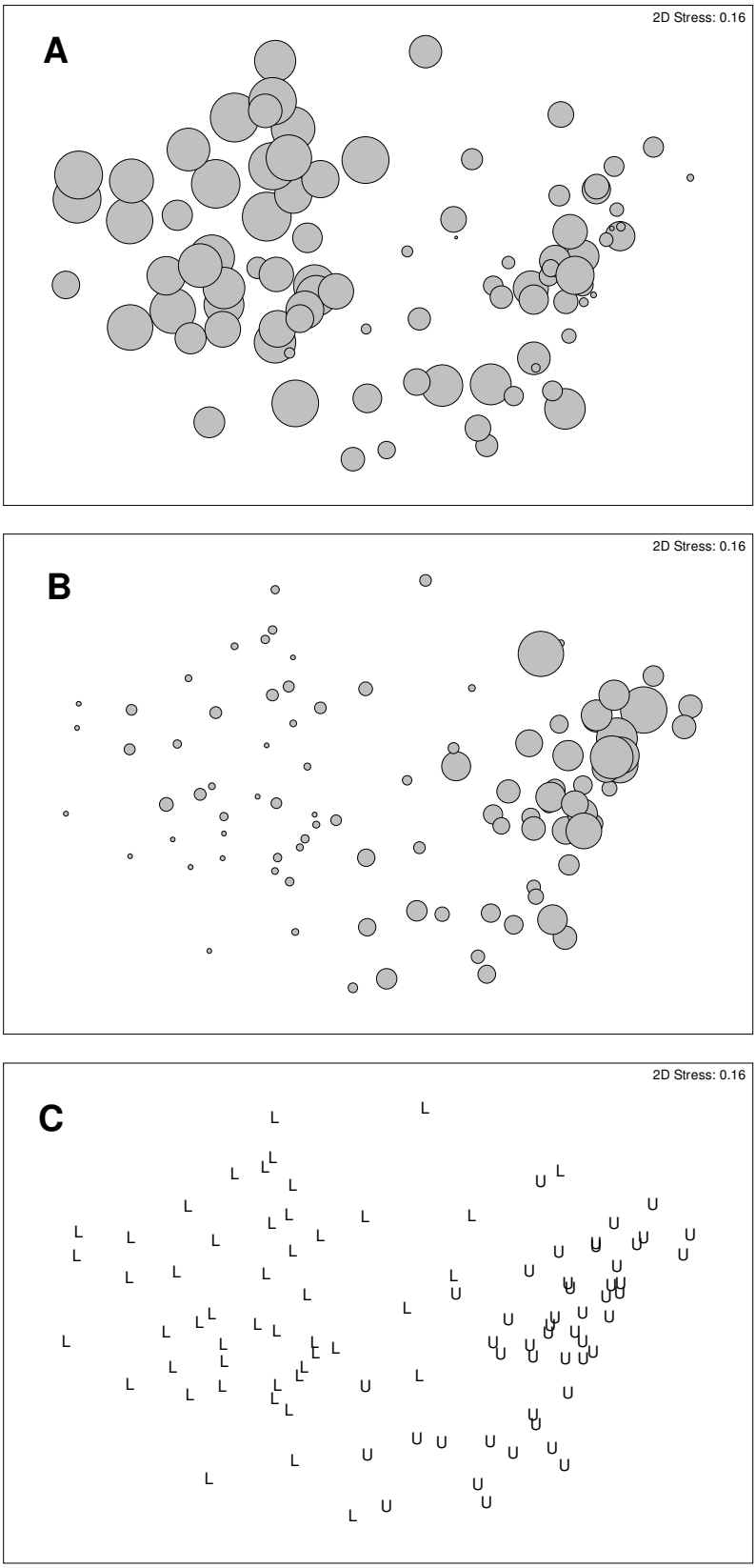

Figure 23. nMDS ordinations displaying bubble plot overlays for A) surface water salinity @ LT, and B) % sediment organic carbon; and C) the position of upper (U) and lower (L) sites in ordination space. Increasing bubble size indicates increasing magnitude of variable for bubble plots.

Summary of results by estuary, time and region

Between estuary patterns

Significant differences between estuaries were found for turbidity, dissolved P, Chlorophyll a and sediment Chl a levels (see summary in table 19). Estuaries also differed in respect to macroinvertebrate species richness, abundance and multivariate assemblage structure. However, generally these differences were inconsistent between estuaries. Water quality measurements such as turbidity, dissolved P and chlorophyll *a* levels were generally highest for the Duck estuary and lowest for the Black estuary. Only turbidity, dissolved P levels and sediment Chl a were higher in the impacted estuaries (Duck and Montagu) compared to the reference estuaries (Black and Detention), although chlorophyll *a* levels were also higher in the impacted estuaries relative to the Black but not the Detention estuary.

In all cases, except chlorophyll *a*, significant statistical interactions between estuary and sampling time (i.e. TxE) were recorded (Table 19). This indicated that detection of differences between estuaries was dependent on the time at which sampling was undertaken. For example, differences between estuaries in terms of nutrients may only be apparent when nutrient concentrations are highest during spring and winter. Attempts to differentiate estuaries in terms of nutrient inputs during the lower river flow period in summer are therefore unlikely to be successful, nor informative.

Macroinvertebrate assemblages displayed a slightly different pattern between estuaries (Table 19). No clear pattern was detected between impacted and reference estuaries. Species richness (no. species) was higher amongst the Montagu estuary, whereas faunal densities were higher in the Montagu and Detention estuaries. Only macroinvertebrate assemblages in the Montagu estuary could be distinguished from the other three estuaries. Analysis of common species individually indicated that this pattern was largely driven by higher numbers of the polychaete *B. limnicola* and the bivalve mollusc *A. semen* in the Montagu estuary relative to the other estuaries. The absence of interactions between estuary and time indicates that this pattern is not contingent on the time of sampling (as is the case with many of the water column variables).

Temporal patterns (seasonal)

A strong seasonal (temporal) pattern was found amongst the water column variables: salinity, turbidity, nutrient and Chl a concentrations, but not amongst the sediment or macroinvertebrate community variables (Table 19). Salinities were highest in summer and autumn, chlorophyll *a* highest in spring and summer and nutrient concentrations highest in winter and spring. Macroinvertebrate assemblage structure displayed a weak temporal pattern (spring differed from the other three sampling dates, see Fig 20) in part driven by a strong seasonal pattern in the abundance of the highly abundant amphipod crustacean *Paracorophium* sp.. The other common invertebrate species considered displayed no temporal patterns in abundance. As discussed above, the time of sampling may alter the outcome of comparisons between estuaries and regions within estuaries.

Table 19. Summary of temporal (T), estuary (E) and regional (R) patterns observed in this study indicating the direction of differences between estuaries and regions for water column, sediment and macroinvertebrate variables. Shaded block indicates where statistically significant effects, including interactions, were recorded. Estuaries: Bl – Black R., Du – Duck R., Dn – Detention R., M – Montagu R.

Variable	Temporal	Estuary	Region	Interactions b/w factors
Water column				
Temperature	Seasonal - Sum/Aut high			
Salinity	Seasonal - Sum/Aut high		Lower>Upper	TxE; ExR
DO				
Turbidity	Weakly seasonal	Du>M>Dn, Bl	Upper>Lower	TxE; ExR
NH_4	Weakly seasonal - Spr/Win high			TxE; TxR
NO_X	Seasonal - Spr/Win high		Upper>Lower	TxE; TxR; ExR
DRP	Seasonal - Spr/Win high	Du>M>Dn>Bl		TxE; ExR
Chl a	Seasonal - Spr/Sum high	Du>Dn, Bl; M>Bl		
Sediments				
Redox				
Organic C			Upper>>Lower	
MPBs (Sed Chl a)		Du, Mn>Dn, Bl	Upper>Lower	ExR
Macroinvertebrates				
Species richness		M>Du, Dn, Bl	Lower>Upper	ExR
Total abundance		M, Dn>Du, Bl		TxR
Assemblage structure	Weakly seasonal?	M ≠ Du, Dn, Bl	Lower ≠ Upper	TxR; ExR
Selected estuarine spp.				
Paracorophium sp.	Seasonal - Spr/Win high		Upper>>Lower	
B. limnicola		M>>Du, Dn, Bl	Upper>>Lower	
A. semen		M>Du, Dn, Bl	Upper>>Lower	
N. australiensis			Upper>Lower	
S. normalis				

Within estuary (between region) patterns

Within estuary differences corresponding with a downstream estuarine gradient were identified for a number of variables in the previous study (Hirst et al. 2005). Several variables in the present study showed clear differences between the upper and lower reaches of the estuaries sampled (Table 19). These included salinity and turbidity (dilution effect); NO_x-N concentrations; sediment organic carbon and chlorophyll *a* levels. Macroinvertebrate species richness and assemblage structure also differed substantially between regions. Salinities and macroinvertebrate species richness were higher in the lower reaches of the estuaries. In comparison turbidity, NO_x-N, Org C and sediment chlorophyll *a* were higher in the upper reaches of these estuaries.

The upper and lower reaches of these estuaries were found to support quite distinct faunal assemblages. The upper estuary assemblages are estuarine in origin and characterised by low species richness, whereas the lower estuary contains a mix of coastal-marine and estuarine species and generally higher species richness. The range of interactions including region (R) indicate that regional differences within estuaries have the potential to confound comparisons between estuaries. Many of the estuary-level differences detected in this study were more pronounced within the upper reaches of the estuaries, including turbidity, NO_x and organic C (see respective sections). It is therefore conceivable that sampling the lower and not the upper reaches of these estuaries will result in the non-detection of these impacts.

Stable Isotope signatures

Stable isotope signatures for tissues extracted from crabs, amphipods and pacific oysters were determined to assess the extent of coupling between anthropogenic sources of nitrogen and food-web components in the Duck and Montagu estuaries. The extent of this coupling was measured relative to the Detention and Black estuaries. In comparison to the Duck and Montagu catchments, human-derived sources of nitrogen into the Detention and Black catchments are low. We would expect that if human-derived nitrogen is an important source of nitrogen in the Duck and Montagu then the $\delta^{15}N$ signatures for these estuaries should be greater than those recorded for the Detention and Black estuaries. This is because anthropogenic sources of nitrogen, such as fertilizer, have higher $\delta^{15}N$ ratios in comparison to natural (background) sources of nitrogen (McClelland and Valiela 1998a,b). Moreover, we would expect higher $\delta^{15}N$ signatures: a) in the upper compared to the lower regions due to reduced tidal flushing, and b) during spring (October) compared to autumn (April) due to higher seasonal inputs of nitrogen. If, in contrast, $\delta^{15}N$ signatures do not differ between estuaries then we can deduce that human-derived sources of nitrogen contribute little to the productivity of the 'impacted' estuaries. This would indicate that a large proportion of the high nutrient loading of nitrogen entering these estuaries is lost via tidal flushing.

Crabs

Three replicate crabs (*Macropthalmus latifrons*) were collected at each site to assess the level of variation between crabs in terms of isotopic signatures. Coefficients of variation (i.e. variance/mean) were calculated for each site and variation was typically low (CVs for $\delta^{13}C$ < 5% and $\delta^{15}N$ <10%). Two-way ANOVA detected differences between estuaries, but not sampling times for C^{13} and N^{15} signatures (Table 20). Post-hoc statistical tests revealed significant differences between all estuaries for $\delta^{15}N$ with the highest values recorded

amongst crab tissues collected from the Duck, followed by the Montagu, Detention and Black estuaries (Fig. 24). By comparison there was much greater overlap between estuaries for δC^{13} signatures (Fig. 24, Table 20).

Table 20 Two-way ANOVA testing for differences in $\delta^{15}N$ and $\delta^{13}C$ signatures between estuaries and sampling dates for *M. latifrons* crabs. Also shown are the outcome and direction of post-hoc tests comparing estuaries.

Source	df	MS	F	P	Post-hoc test
$\delta^{15}N$					
Estuary	3	25.39	93.13	0	Du>M>Dn>Bl
Season	1	0.01	0.02	ns	
Estuary*season	3	0.40	1.47	ns	
Residual	58	0.27			
$\delta^{13}C$					
Estuary	3	3.44	4.22	0.009	M>Du
Season	1	0	0	ns	
Estuary*season	3	0.20	0.24	ns	
Residual	58	0.82			

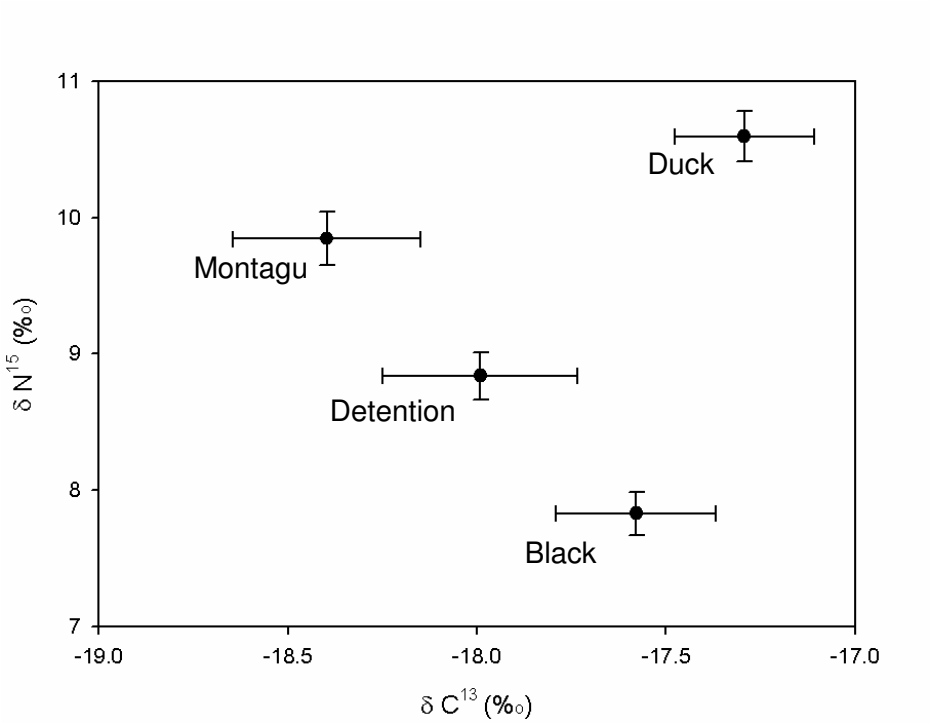

Figure 24. Mean (±SE) carbon and nitrogen isotope values for crab tissue collected from three locations in each of the four estuaries pooled across sampling dates (n = 18 individuals per estuary).

The pattern for δN^{15} values is broadly consistent with known nitrogen loadings for these four estuaries (see table 1). Nitrogen loadings in the Duck and Montagu estuaries are amongst the highest in the state (Bobbi et al. 2003) and are assumed to be high relative to the Detention and Black estuaries due to the absence of intensive dairy farming in these two catchments.

Amphipods

A similar pattern was also found for amphipods collected in October 2006. N^{15} signatures for the Montagu and Duck estuaries were higher relative to the Detention and Black estuaries, although only the Montagu estuary values were significantly higher ($F_{3,9} = 7.66$, $P = 0.018$) (Fig. 25). Isotopic values of carbon showed no differences between estuaries ($F_{3,9} = 1.54$, $P>0.05$). Doubtless, the statistical power of this comparison could be improved by increasing either the number of replicate sites sampled per estuary (i.e. n > 3 sites) or the number of replicate samples per site. However, this study was constrained by collecting sufficient numbers of animals in the field to supply ample sample (when dried) for stable isotope analysis.

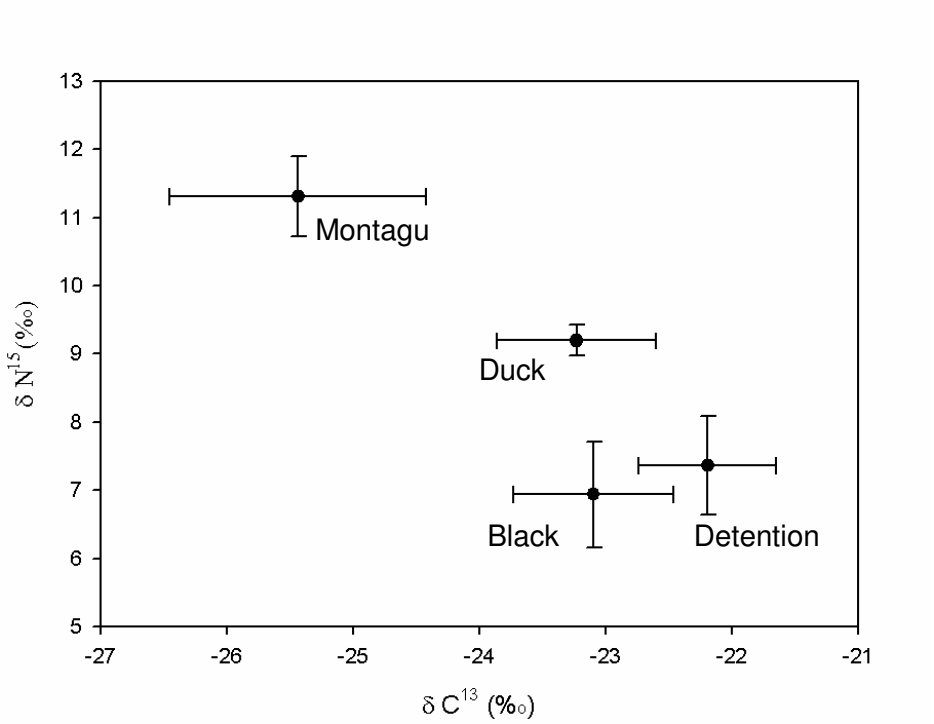

Figure 25. Mean (±SE) carbon and nitrogen isotope values for amphipods collected from three locations in each of the four estuaries in October 2006 (n = 3 samples per estuary).

Oysters

By comparison, oysters collected from the lower reaches of the estuaries displayed no such pattern (Fig. 26). The highest N^{15} signatures were found amongst oysters collected from the Duck, Black and Detention estuaries; whereas oysters from the Montagu estuary could be consistently distinguished from these three estuaries in terms of their N^{15} and C^{13} signatures (Table 21). As with the crab tissue, CV for individual oysters collected within sites was low (oyster muscle tissue CVs for $\delta^{13}C$ and $\delta^{15}N$ <10%).

Table 21 Two-way ANOVA testing for differences in $\delta^{15}N$ and $\delta^{13}C$ signatures between estuaries and sampling dates for *C. gigas* oyster muscle tissue. Also shown are the outcome and direction of post-hoc tests comparing estuaries.

Source	df	MS	F	P	Post-hoc test
$\delta^{15}N$					
Estuary	3	6.365	20.215	<0.001	Bl,Dn,Du>M
Season	1	0.074	0.235	ns	
Estuary*season	3	0.017	0.053	ns	
Residual	64	0.315			
$\delta^{13}C$					
Estuary	3	24.075	21.925	<0.001	Du>Bl,Dn,M; Dn,Bl>M
Season	1	0.015	0.014	ns	
Estuary*season	3	0.885	0.806	ns	
Residual	64	1.098			

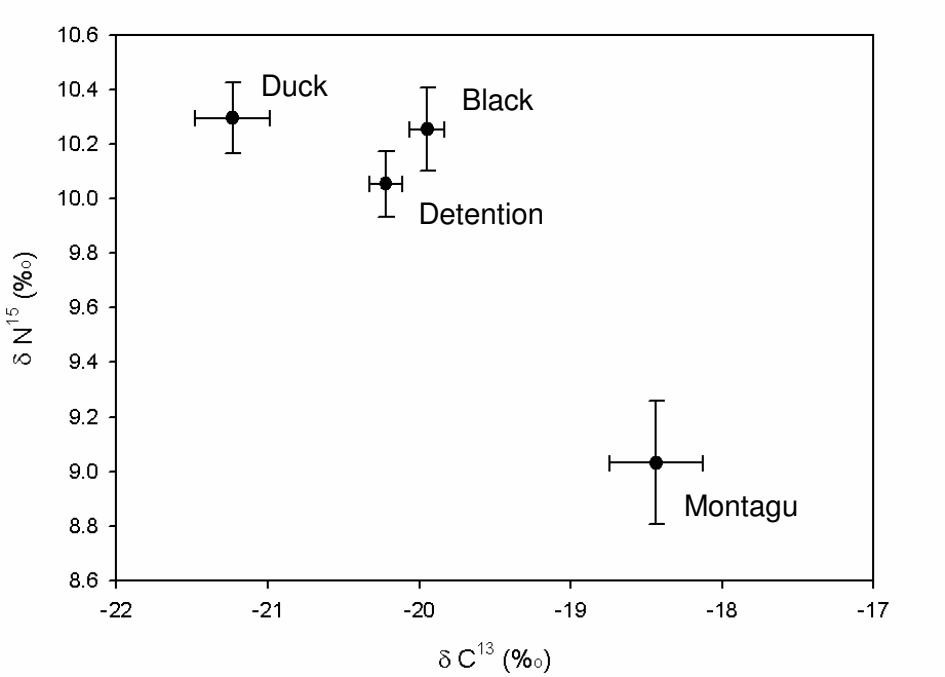

Figure 26. Comparison of mean (±SE) carbon and nitrogen isotope values for oyster tissues collected from three locations in each of the four estuaries (n = 18 individuals).

Sediments

In the design presented in table 2 sediments were collected from the upper and lower regions of each estuary. However, sediments collected from the lower region (invariably sands) contained such low levels of nitrogen that strong isotopic signatures of nitrogen were difficult to detect (i.e. < 0.05%). Consequently, sediments from the lower regions were omitted during analysis. Two-way ANOVA detected significant differences between estuaries, but not sampling times. Post-hoc Tukeys tests revealed that δN^{15} signatures in the Montagu and Duck estuaries were significantly enriched compared to those recorded amongst sediments collected from the Detention and Black estuaries (Tukey HSD test $P < 0.001$). As with the crab tissue and the amphipods, δN^{15} signatures were lowest in the Black estuary.

Table 22 Two-way ANOVA testing for differences in δN^{15} signatures between estuaries and sampling dates for sediments. Also shown are the outcome and direction of post-hoc tests comparing estuaries.

Source	df	MS	F	P	Post-hoc test
Estuary	3	6.692	18.229	<0.001	M,Du>Dn,Bl
Season	1	0.135	0.368	ns	
Estuary*season	3	0.247	0.673	ns	
Residual	16	0.367			

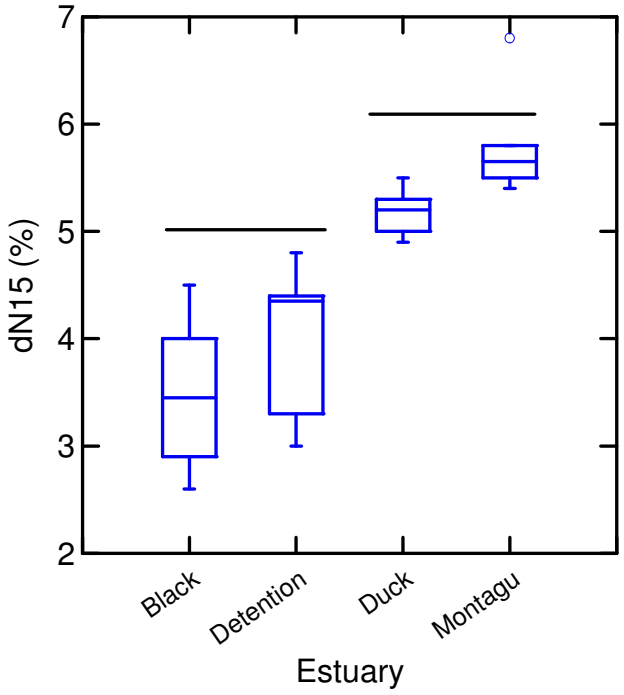

Figure 27 Boxplots display range of δN^{15} values for sediments collected in the upper regions of each of the four estuaries (pooled across sampling times). Horizontal lines are medians, boxes are interquartiles and vertical bars are ranges excluding outliers (°). Horizontal bars indicate groups that are significantly different at $P < 0.001$ following Tukeys HSD post-hoc test (see table 22).

Discussion

Assessment of status of the Duck and Montagu estuaries

No consistent pattern emerged in response to catchment impacts among these estuaries. The Duck and Montagu estuaries had higher turbidity, dissolved reactive phosphorous and sediment chlorophyll *a* levels, but only the Montagu could be distinguished from the two reference estuaries in terms of benthic community structure (see Table 23). The differences in water column variables and MPB levels shown in table 23 are consistent with what is know about the state of the Duck and Montagu River catchments. High nutrient loads and associated catchment inputs translated into higher ambient dissolved phosphorous and turbidity levels, but not, as might have been expected, higher ambient levels of NO_x or NH_4. Higher nutrient loadings in turn appeared to stimulate higher algal productivity amongst the impacted estuaries. Algal biomass amongst the benthos was approximately 60% higher in the Duck and Montagu estuaries relative to the reference estuaries and a similar, although less strong, pattern was also evident for water-column algal biomass. Nonetheless, evidence of detrimental human-induced impacts in these estuaries was difficult to find. Dissolved oxygen levels were within acceptable limits (see fig. 9) and there was no evidence of organic enrichment of the benthos. The macroinvertebrate fauna surveyed provided little indication that the sediments were severely organically enriched, supporting an abundant fauna of estuarine specialists typical of the upper reaches of estuaries in SE Australia (see Table 16) (Edgar et al. 1999; Hirst 2004).

Table 23. Sub-set of statistically significant outcomes between estuaries taken from table 19.

Variable	Estuary
Water column	
Chlorophyll a	Du>Dn, Bl; M>Bl
Turbidity	Du>M>Dn, Bl
DRP	Du>M>Dn>Bl
Benthic	
MPBs	Du, Mn>Dn, Bl
Macroinvertebrates	
Assemblage structure	M ≠ Du, Dn, Bl
Total abundance	M, Dn>Du, Bl
B. limnicola	M>>Du, Dn, Bl
Species richness	M>Du, Dn, Bl
A. semen	M>Du, Dn, Bl

The macroinvertebrate communities in the Montagu estuary had higher overall species richness, higher total abundances than the Duck and Black estuaries and higher abundances of the spionid polychaete *B. limnicola* and the bivalve mollusc *A. semen* than the other estuaries. Higher species richness is contrary to expectations of how coastal ecosystems respond to elevated levels of nutrients. Rather, the high species richness of the Montagu estuary is more likely to be related to the proximity of the lower reaches of the Montagu to the complex array of intertidal/subtidal soft-sediment habitats found in Robbins Passage. By comparison the upper reaches of the four estuaries were remarkably similar in terms of invertebrate

composition, with the exception of high densities of *B. limnicola* present in the Montagu estuary. *B. limnicola* is an estuarine specialist that is known primarily from locations with low salinities (Blake and Woodwick 1976). This species may also be indicative of organically enriched habitats (as are many spionid polychaetes), but it is unclear why is it only restricted to the upper reaches of the Montagu and not the Duck estuary, which by all other measures is equally impacted.

Whilst there is clear evidence of lower water quality in the Duck and Montagu estuaries, in terms of higher dissolved P levels, turbidity and to a lesser extent algal productivity, this does not appear to translate into any tangible impacts: no significant changes in oxygen levels, sediment chemistry or macroinvertebrate community composition were detected. Thus, these estuaries appear to be in reasonably good health despite the poor condition of their respective catchments (Horner et al. 2003; Pinto et al. 2003). Despite high nutrient inputs during winter-spring, chlorophyll *a* levels only exceeded the ANZECC water quality guidelines for estuaries (ANZECC 2000) during the height of summer (January sample), and then only marginally. Moreover, the statistical interaction terms indicated that many of the detected impacts (see summary above) were only manifested in the upper reaches of these estuaries and during periods of higher river flows. Impacts are therefore variable in space and time, diluting their overall effect on the estuarine ecosystems. There is little doubt that high tidal flushing (leading to lower nutrient residence time) plays an important role in mitigating these impacts. This conclusion is supported by the fact that impacts tend to be 1) restricted to the upper reaches of the estuaries where tidal exchange is lower and stratification higher; and 2) more prominent in spring/winter when river inputs are higher increasing overall residence time. Hence these estuaries appear to relatively resilient to the catchment derived inputs they receive.

It is worth noting that this study was undertaken during one of the driest winters on record (2006). Therefore, whilst spring, summer and autumn measurements are likely to fall within the normal range of values expected in these estuaries, winter measurements may be quite anomalous (see also fig. 5). It is conceivable that more exaggerated impacts, may have been recorded had the sampling been undertaken during normal winter conditions. However, it is unclear whether this would have resulted in greater changes to the benthic invertebrate fauna. Fluctuations in the abundance of the amphipod *Paracorophium* sp. aside, macroinvertebrate assemblage structure appeared to be remarkably stable throughout the duration of this study and many of the common species appear to be unaffected by changes in freshwater flows and salinity. Holz (2005) found that groundwater inputs of nutrients into the Montagu catchment from dairy pasture coincided with periods of high rainfall that flushed accumulated nitrates and phosphorous from the soil. The highest nutrient inputs were recorded in June following the first major rainfalls; followed by smaller peaks throughout the remainder of winter and spring. Inputs in summer and autumn were minimal by comparison. Hence, the majority of nutrient inputs in this catchment appear to occur during the wetter, colder months when the risk of eutrophication is reduced due to lower productivity.

As discussed earlier (see introduction) this assessment is predicated on the choice of suitable reference points - in this case the Black and Detention river estuaries. The Black estuary consistently had the 'best' water quality in terms of dissolved phosphorous, turbidity and chlorophyll *a* levels of the four estuaries. The Detention estuary had similarly high water quality by comparison, although dissolved phosphorous levels were found to be marginally higher. Although the Detention River catchment is not as modified as the Duck and Montagu catchments, wholesale clearing of riparian vegetation on the eastern bank of the upper reaches

of this estuary (cleared in spring 2004; A. Hirst pers. obs.) may raise concerns about its suitability as a reference estuary. Nonetheless, in all other respects, the results of this study validated the choice of the reference estuaries used.

Efficacy of indicator variables

This study found that the detection of impacts in estuaries was contingent on both the spatial and temporal scales at which variables were measured. This was the case for the water quality variables: salinity, turbidity, dissolved nutrients and sediment chlorophyll *a*, which all displayed significant statistical interactions between estuary and time and estuary and region (see Table 19). That is the detection of differences between estuaries was dependent on the both the time and location of sampling. Invariably, significant differences between estuaries were restricted to the wetter months (with the exception of chlorophyll *a* levels) and/or the upper reaches (region) when and where impacts were most exaggerated. Failure to sample the appropriate temporal and spatial scales in these estuaries will therefore result in monitoring studies concluding erroneously that no impacts occur when in fact the opposite maybe true.

By comparison benthic invertebrate assemblages were far less variable in time, despite substantial changes in salinity and other abiotic variables. Hirst et al. (2005) found a similar lack of variation between assemblages sampled in spring and autumn. Temporal changes only explained a very small percentage of the overall variation in assemblage structure (see Table 14). This suggests that macroinvertebrates may be a more robust measure of estuarine condition than water quality parameters. The only temporal changes of significance were found for the amphipod *Paracorophium* sp., whereas many of the other common species displayed little variation over time. The downside to this stability, is that estuarine macroinvertebrates are unlikely to be useful indicators of minor perturbations/impacts (that are ordinarily difficult to distinguish from natural variability in any case), but may provide greater certainty in the detection of moderate to major perturbations. Change in the composition of macroinvertebrate communities may also provide clues about the nature of the impacts.

The upper and lower estuarine regions were found to support quite distinct faunal assemblages displaying little overlap in species composition. The upper estuary fauna comprise estuarine specialists, largely restricted to estuaries and capable of tolerating a wide array of salinities, whereas the lower regions contains marine stenohaline and coastal species with some estuarine species present at lower densities. As catchment impacts appear to be manifested to a greater degree in the upper estuary we recommend sampling macroinvertebrate communities in this part of the estuary. Changes to these ‘upper estuarine’ invertebrate communities are likely to tell us more about changes to the condition of the estuary (be they anthropogenic or natural) than those communities found at the mouth and in the lower parts of the estuary. This is because changes to the latter are likely to reflect, or be confounded by, recruitment from adjacent coastal areas, independent of changes within the estuary – although this contention is largely untested at this point. One obvious exception to this recommendation is when changes to the opening (and thus closing) regime of an estuary mouth is considered. In this case sampling of the invertebrate communities within the lower estuary would provide important information. If seasonal sampling cannot be undertaken we would recommend sampling in spring. Samples collected in spring provide information on estuarine communities following a period of typically higher river flows and freshwater inputs and, thus, potentially higher inputs of nutrients and contaminants originating from within the

catchment. This also coincides with the optimum period for sampling water quality (see above). However, if possible more than one season should be sampled to increase certainty.

Salinity, DO and turbidity can be measured simply and reliably in the field using electronic probes. Similarly measurements of dissolved nutrients and chlorophyll *a* levels can be made by collecting water samples in the field and sending them to an accredited laboratory for analysis with only minimal processing in the field. Sediment samples for C, N and other contaminants can also be collected and analysed by a number of accredited analytical laboratories. In contrast, measurements of microphytobenthic (MPB) biomass and macroinvertebrate community structure require expertise that is only likely to be available through specialist research institutes. However, we reiterate that the measurement of sediment-based variables is likely to be essential in monitoring tidal estuaries like those found in north-west Tasmania because these variables are less likely to be influenced by short-term fluctuations in tidal cycles and/or river inputs. MPB biomass was found to consistently distinguish estuaries and regions (Table 19). Sediment redox measurements in the field were highly variable, displaying no consistent pattern between the upper and lower reaches even when the sediment organic carbon and MPB data indicated there should have been detectable differences. From personal experience, redox is difficult to measure accurately and consistently in the field and based on the findings of this study we would not recommend measuring redox.

Stable isotope signatures for estuaries with differing anthropogenic N inputs

In Australia, Stable isotope (SI) methods have rarely been used at a multi-catchment (estuary) scale to address questions pertaining to the impacts of anthropogenically-derived nutrient inputs into estuaries. Rather, SI methods have generally been tested by validating them against clear, but localised, disturbance gradients (e.g. Gaston et al. 2004; Piola et al. 2006). The challenge is to apply these methods at spatial scales relevant to the management of catchments, estuaries and coastal systems (although see Moore and Suthers 2005 for a recent example of this approach in NSW). Understanding the extent of linkages between anthropogenic sources of nitrogen and productivity in estuarine food webs is a precursor to anticipating risk associated with anthropogenic inputs and directly feeds into informed management of estuaries and their associated catchments.

Using a range of estuaries on the east coast of the US with differing levels of anthropogenic N inputs, Martinetto et al. (2006) found that the $\delta^{15}N$ of dissolved inorganic nitrogen entering estuaries increased with increasing N inputs. Furthermore, higher $\delta^{15}N$ signatures were consistently reflected throughout the aquatic food-webs (e.g. aquatic macrophytes, sediment, benthic invertebrates, fishes and zooplankton) of estuaries with higher N inputs. In the current study we found higher $\delta^{15}N$ signatures in crabs, amphipods and sediments (in the upper reaches) in the putatively impacted estuaries, but not in Pacific oysters. Contrary to initial expectations there were no seasonal differences in $\delta^{15}N$ or $\delta^{13}C$ signatures for any of the target groups.

The important question is how ecologically meaningful are these differences in $\delta^{15}N$ between impacted and reference estuaries? In the Martinetto et al. (2006) study, the least impacted catchment received 14 kg N ha^{-1} yr^{-1}, whereas the most impacted catchment received 600 kg N ha^{-1} yr^{-1}, much of this extra load originating from anthropogenic sources of N (McClelland and Valiela 1998). The difference in $\delta^{15}N$ between the estuaries with the highest and lowest N inputs averaged across all decapod crustaceans (crabs, shrimps etc.) collected in the

Martinetto et al. (2006) study was 2.72‰. In our study the difference between burrowing crabs in the Duck and Black estuaries was 2.76‰ (see fig. 24). Hence the differences in the NW estuaries are at least comparable in magnitude to those examined in the US study. The differences were even greater for the benthic amphipods examined in this study. Amphipods collected from the upper reaches of the Montagu estuary were enriched in ^{15}N by 4.37‰ relative to the Black estuary (fig. 25) compared to a mean difference of 3.5‰ for deposit feeding invertebrates examined in the Martinetto et al. (2006) study. Sediments in the Montagu estuary were enriched by 2.3‰ relative to the Black estuary, compared to 2.7‰ in the Martinetto et al. (2006) study. These results indicate that anthropogenically-derived nitrogen is an important contributor to benthic food-web productivity in the upper reaches of the Duck and Montagu estuaries. The Black estuary consistently recorded the lowest $\delta^{15}N$ signatures of the estuaries surveyed in line with initial expectations of this being the least impacted estuary of the four (see introduction).

By comparison the absence of estuary differences for Pacific oysters is consistent with the location and trophic ecology of this species. Dilution of catchment-derived N is likely to be greater in the lower reaches of the estuaries – where oysters primarily reside – compared to the upper reaches. In addition, oysters are intertidal filter-feeders that feed on water-borne material when submerged and are thus largely dependent upon food items that arrive during an incoming high tide, deriving their nitrogen from primarily coastal sources. Other studies have found that oysters and other filter-feeding bivalves are useful indicators of localised (Piola et al. 2006) and catchment-wide disturbance (Moore and Suthers 2005). Thus, the absence of an effect amongst of these estuaries cannot be attributed to the fact that oysters are poor indicators of nitrogen enrichment, but that the food-webs in the lower reaches of these estuaries are largely uncoupled from catchment-derived inputs of nitrogen.

Ecology and conservation status of tidal-river estuaries in NW Tasmania

Estuaries are typically dynamic environments displaying high variation over short- (tidal) and longer-term (seasonal) temporal scales. Estuaries in NW Tasmania are characterised by both high tidal exchange and highly seasonal river flows that accentuate such physical variation. This and the previous study have shown marked changes between tidal cycles (see Hirst et al. 2005) and seasons (see table 19) for water-column variables such as salinity, turbidity, nutrient and chlorophyll *a* levels. By comparison, similar temporal patterns were conspicuously absent from the benthos/sediments (see table 19, but also Hirst et al. *in review*). The water-column environment is therefore substantially more dynamic than the benthos and this has clear implications for monitoring estuaries in NW Tasmania (see above).

All estuaries examined in this study exhibited clear environmental and biological gradients extending from the upper reaches of the estuary to the marine mouth. This pattern is best illustrated in Hirst et al. (2005) where a greater number of sites were sampled per estuary. In general terms, the upper reaches of these estuaries are dominated by finer, organically enriched sediments, lower salinities, higher turbidity and nutrient levels and support a restricted fauna of estuarine species that usually exist at higher densities (see table 16). The lower and marine reaches of the estuaries are dominated by sandier, coarser sediments, higher salinities, lower turbidity and nutrient levels and range of invertebrate species also commonly found along the coast. Sediments in the upper reaches may also be more productive, supporting marginally higher levels of microalgal biomass in this study (see table 19). Published information on spatial distribution of microphytobenthos in estuaries often shows correlations with nutrient gradients (Underwood and Krokamp 1999). Finer (muddier)

sediments usually contain a higher organic content (see results), whereas sandier sediments tend to be more nutrient poor (Underwood and Krokamp 1999).

This gradient is shaped by the high tidal incursion experienced by these estuaries. During summer when river flows are low, tidal influence may extend up the river by 3–4 kilometres. Progressively the influence of the incoming tide is diminished as it moves up into the estuary, mixing with river waters, creating a gradient from marine to freshwater in terms of salinities and other water-quality parameters. This gradient contributes to the overall biodiversity of these estuaries by creating range of environments capable of supporting estuarine, coastal and marine species. The lower reaches of these estuaries supported a higher diversity of invertebrate species than the upper reaches (see table 11). This is because benthic communities in the lower reaches comprise species drawn from more diverse adjacent coastal environments, whereas the upper communities comprise a limited array of estuarine specialists that are often restricted to upper reaches of estuaries (as defined in this report). In general, the Duck and Montagu estuaries had higher overall species counts than the Black and Detention estuaries due to higher numbers of species recorded from the lower, but not upper reaches. This probably stems from the fact that the lower reaches of the Duck and Montagu estuaries are larger in comparison to the Black and Detention estuaries (see Fig. 3), supporting a larger diversity of habitats and consequently a bigger pool of coastal species.

As part of a broad-scale assessment of 111 estuaries across Tasmania, Edgar et al. (1999) identified estuaries of critical regional and conservation significance, largely on the basis of the status of the catchment. The Black river estuary was assigned the highest conservation status (Class A) of any estuary in north-west Tasmania (Class A) because it has the least modified catchment in the region. The Detention and Montagu estuaries were assigned moderate conservation significance (Class C) because their catchments have been affected by human habitation and clearance, but not considered overly degraded. The Duck estuary was assigned the lowest conservation significance of the four estuaries (Class D – moderately degraded) because the associated catchment is moderately degraded.

Based on the information obtained in this study we believe revision of these assessments is warranted. First, there is no justification for assigning the Duck and Montagu estuaries a conservation status based simply on the state of their respective catchments. While our study found reduced water quality in the upper regions of these estuaries, impacts in the form of predictable changes to biological communities, relative to the reference estuaries, were difficult to find. Furthermore, the lower regions of these estuaries supported a greater diversity of invertebrate fauna than the reference estuaries. Duck Bay and Robbins Passage, adjacent to the mouth of the Montagu River, also comprise large areas of intertidal sand flats, punctuated by a myriad of tidal drainage channels that provide a complex array of habitats for sea and wading birds. The proximity of Robbins Passage gives the Montagu estuary system special significance. Hence we would argue that the Duck and especially the Montagu estuary should receive higher conservation significance than they have been currently assigned by Edgar et al (1999). We would concur with Edgar et al.'s (1999) initial classification of the Black River estuary – and this estuary consistently had the best water quality of the four estuaries (see table 19). On the other hand the conservation status of the Detention has been diminished by recent changes to riparian vegetation bordering the upper reaches (see comments above). While serving as a useful reference point in this study, the conservation significance of this estuary may not be particularly high due to its relatively smaller size and proximity to the township of Hellyer.

Conclusions

Whilst there was clear evidence of lower water quality in the Duck and Montagu estuaries, in terms of higher dissolved phosphorous levels, turbidity and to a lesser extent water-column and sediment based algal productivity (particularly in the upper reaches), this translated into few discernable impacts. There were no significant changes in oxygen levels, sediment chemistry or macroinvertebrate community composition. Thus, these estuaries appeared to be in reasonably good health despite the poor condition of their respective catchments.

When selecting appropriate indicators, greater emphasis should be given to benthic processes and variables over water-column variables in these estuaries. In general these indicator variables displayed less variability than the water-column variables and the benthos is known to be an important site for the storage and processing of nutrients within estuaries.

Benthic invertebrate assemblages were found to be relatively stable through time, despite major changes in salinity and other abiotic variables. This suggests that macroinvertebrates may be more robust measures of estuarine condition than water quality parameters, with only limited sampling in time required to detect moderate to major perturbations.

Anthropogenic sources of nitrogen such as those originating from the use of fertilizer in dairy pastures have distinct (and elevated) isotopic signatures. Stable isotope signatures of deposit-feeding invertebrates and sediments collected from the upper reaches of these estuaries were more enriched the impacted (Duck and Montagu) compared to the reference estuaries (Detention and Black), but this was not the case for Pacific oysters collected from the lower reaches. While there is evidence that food-webs in the upper reaches were linked to terrestrially derived sources of nitrogen, food-webs in the lower reaches remain largely uncoupled from these sources. This concurs with the finding that impacts, where detected, were primarily restricted to the upper reaches of these estuaries.

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Appendices

Appendix 1. Latitude and longitude (decimal degrees) for 24 sites surveyed in this study.

Estuary	Region	Site	Latitude	Longitude
Black	Lower	BL1	40.837931	145.3171618
Black	Lower	BL2	40.839481	145.3111422
Black	Lower	BL3	40.842796	145.3094793
Black	Upper	BU1	40.846455	145.3092487
Black	Upper	BU2	40.84741	145.3056977
Black	Upper	BU3	40.847168	145.3014975
Detention	Lower	DNL1	40.875449	145.4466165
Detention	Lower	DNL2	40.88187	145.4457684
Detention	Lower	DNL3	40.885909	145.4477748
Detention	Upper	DNU1	40.8882	145.43954
Detention	Upper	DNU2	40.8889	145.43689
Detention	Upper	DNU3	40.89044	145.43525
Duck	Lower	DUL1	40.796099	145.1090006
Duck	Lower	DUL2	40.796448	145.1020425
Duck	Lower	DUL3	40.806758	145.1125947
Duck	Upper	DUP1	40.834085	145.122325
Duck	Upper	DUP2	40.840061	145.114053
Duck	Upper	DUP3	40.842506	145.1079059
Montagu	Lower	ML1	40.75006	144.94307
Montagu	Lower	ML2	40.74839	144.93621
Montagu	Lower	ML3	40.752846	144.9318508
Montagu	Upper	MU1	40.768886	144.9242222
Montagu	Upper	MU2	40.767555	144.929415
Montagu	Upper	MU3	40.771734	144.9308422

Appendix 2. Presence/absence of macrobenthic fauna collected from each estuary October 2005–July 2006. + signifies total abundance <10, ++ > 10 and +++ >100 individuals. Blanks signify taxa absent from estuary.

Taxa		Black	Detention	Duck	Montagu
Crustacea					
Amphipoda	Dexaminid sp.			+	
	Gammaropsis sp.	++	++	++	++
	*Limnoporeia yarragu*e	+	+	+	++
	Lysianassid sp.				+
	Melitidae sp.	+			+
	Paracalliope sp.		+	++	++
	Paracorophium sp.	+++	+++	+++	+++
	Phoxocephalid spp.			++	++
	Tethygeneia sp.	+	++	+	+
	Urohaustorius halei	++	+	+++	++
Cumacea	Cumacea sp. A		+	++	++
	Dimorphostylus colefaxi	+	+	+	+
Decapoda	*Amarinus* spp.		++	+	++
	Bellidillia laevis				+
	Biffarius spp.	+++	+	++	+
	Heloecius cordiformis			+	+
	Macrophthalmus latifrons	+	+	+	
	Mictyris platycheles	++	++	++	+
	Paragraspus gaimardii	+	+		
Isopoda	Anthuridae unid.				+
	Cirolanidae sp.			+	+
Mysidea	Mysidae spp.	+	+	+	++
Tanadicea	Tanaidae unid.	+			++
Insecta					
	Chironomidae spp.	++	+++	+	+++
Mollusca					
Bivalvia	*Anapella cycladea*	++			
	Arthritica semen	+++	+++	+++	+++
	Katelysia rhytiphora	1			
	Katelysia scalarina	++	++	+	+++
	Mysella donaciformis	++	++	+++	+++
	Paphies elongata			+	+
	Paphies erycinea	+++	+++	+++	+++
	Solemya australis				++
	Soletellina biradiata	+		+	+
	Tellina deltoidalis	+	+	+	
Gastropoda	*Agatha metcalfei*			+	
	Ascorbis victoriae		+		++
	Hydrococcus brazieri	++	+	+	+++
	Nassarius spp.	++	++	++	++
	Patelloida insignis			++	+
	Polinices conicus	+		+	
	Salinator sp.	+	++	+	+
	Tatea rufiabris			+	+
Polychaeta	*Anoides oxycephala*			+	+
	Aricidea pacifica	+			+
	Armandia sp.				+
	Boccardiella limnicola	+	++	++	+++

	Capitella spp.	++	+	+	+
	Dipolydora pencillata	+	+	++	++
	Dorvillea sp.			+	
	Euzonus sp.		+		+
	Glycerid sp.				+
	Hesionidae sp.			+	
	Lumbrinereis sp.	+	+	+	+
	Magelona sp.	++	+	+	
	Microspio granulata	+	+	++	++
	Nephtys australiensis	+++	+++	+++	+++
	Nephtys longipes	+	+	++	
	Olganereis edmondsi			+	++
	Paraonidae sp.	+			
	Phyllodoce sp.	+	+	+	+
	Scoloplos normalis	+++	+++	++	++
	Scoloplos simplex	+	+		++
	Simplisetia aequisetis	+	+	++	+++
	Travisia sp.				+
Nemerteans	Nemerteans unid.	+	+	+	
Cnidiaria	*Edwardsia* sp.				+
Sepuncula	*Phascolosoma annulatum*		+	++	