

Spatial variation in the macrobenthic assemblages of intertidal seagrass along the long axis of an estuary

Authors: R.S.K. Barnes, M.D.F. Ellwood

Abstract

Invertebrate macrofaunal biodiversity within intertidal seagrass meadows was investigated over a salinity gradient of $5-35$ in the Knysna estuarine system (Garden Route National Park, South Africa). Rather than the classic gradual decline in species richness with distance from the mouth there were zones of considerable faunal stability separated by relatively sharp discontinuities. At the point upstream at which salinity regularly falls below 30, the rich, diverse and highly spatially variable downstream faunal assemblage changed to a less rich, less diverse and more uniform one that dominated the upstream stretch without any further upstream reduction in richness. Nevertheless, without loss of overall richness, assemblage composition changed, again rapidly, in the upper region of the upstream stretch to a zone dominated by the microgastropod *Hydrobia*, which otherwise occurs in the Knysna system only in highly sheltered regions of the downstream stretch where it is also dominant. The upstream faunal assemblage was a subset of that in the marine-influenced downstream region not a different replacing one. Position along the estuarine gradient accounted for 29% of total assemblage variation. Overall faunal abundance declined with distance upstream until the *Hydrobia* zone where it rose sharply, but there was no evidence of increase in density of those species remaining on putative release from competition.

1. Introduction

Ever since the classic papers of Remane (1934) and Alexander et al. (1935), organisms that occur in estuarine systems have been portrayed largely as a series of predominantly marine species that penetrate into brackish waters to differing degrees, each dependent on its tolerance limit to lowering salinity — or more likely to the degree of salinity fluctuation (Montague and Ley, 1993; Attrill, 2002) — with the consequence that species richness continually declines with increasing distance upstream until the onset of freshwater conditions (Cognetti and Maltagliati, 2000; Attrill, 2002; Attrill and Rundle, 2002; Whitfield et al., 2012). Although this ‘Remane paradigm’ (Remane, 1934, 1971) has become ‘the recognized textbook model for diversity patterns in tidal estuaries’ (Attrill, 2002) and ‘central ... in estuarine ecology’ (Whitfield et al., 2012), it is clearly subject to a number of qualifications when applied to estuaries (Day, 1981; Attrill, 2002; de Biasi et al., 2003; Teske and Wooldridge, 2004; Whitfield et al., 2012). One potential caveat is that the occurrence of the phenomenon in estuarine benthos has been based on data from, e.g. unvegetated soft sediments (Schlacher and Wooldridge, 1996), rocky outcrops (Bassindale, 1943) or the whole benthic system (Rodrigues et al., 2011), but never specifically on the benthic faunal assemblages supported by seagrass. Earlier work (Barnes and Ellwood, 2011; Barnes and Barnes, 2012) has shown that the equivalent textbook paradigm of declining species richness and diversity with progression from the sublittoral up the intertidal zone applies poorly within stands of two seagrasses, *Nanozostera capensis* and *Nanozostera muelleri*, possibly in some measure because the vegetation mitigates the usual effects of decreasing tidal cover on sediment-dwelling animals. Correspondingly, the presence of seagrass along an estuarine axis might, for example, have an additional dampening effect on the already reduced salinity variation within the sediment compared to that in the overlying water (Alexander et al., 1932; Kinne, 1967) and thereby also affect distributions. In the light of this, the manner and extent to which faunal richness and diversity within intertidal estuarine seagrass beds decrease upstream is open to question, as is the degree of change within seagrass faunal assemblages along the upstream gradient.

Notwithstanding that much work has been done on the importance of both salinity and seagrass to estuarine faunal abundance and diversity (e.g. Collett et al., 1984; Edgar and Barrett, 2002), it appears that the sole study partly to address these questions has been that of Yamada et al. (2007) on the Akkeshi-ko system in northern Japan, although this investigation was only semi-quantitative and confined to the seagrass epibenthos. Concentrating on beds of the subtidal *Zostera marina*, these authors did find a significant relationship between salinity and species richness of epifaunal malacostracans and gastropods (the only taxa studied) in the seagrass. But they found little evidence of different species assemblages occurring in different regions of the gradient, and there was no relationship between overall faunal abundance and

salinity. This combination of results could be taken to indicate that faunal assemblages in the upstream regions of estuarine seagrass may be little more than impoverished versions of those in more marine areas, with a few, particularly tolerant species reacting there to their release from competition by increasing in density.

The dominant seagrass in southern Africa is Cape dwarf-eelgrass, *N. capensis*, which is particularly associated with estuarine conditions (Bandeira and Gell, 2003; Whitfield et al., 2012). Its South African stronghold is the estuarine bay (sensu Whitfield, 1992) at Knysna, known locally as the Knysna Lagoon, within the Garden Route National Park (Russell et al., 2009). This system supports 350–390 ha of *N. capensis* (Maree, 2000; Bandeira and Gell, 2003); i.e. over half of the total national area. Like all *Nanozostera* species, *N. capensis* can certainly tolerate low salinity, down to some 2 (Iyer and Barnabas, 1993), and hence its usual categorisation as being estuarine in habitat. Nevertheless, like many ‘estuarine’ organisms (Barnes, 1989; Attrill and Rundle, 2002), dwarf-eelgrasses are probably more truly characteristic of sheltered coastal conditions in general and they occur in areas other than those of reduced salinity. Indeed they flourish in full-strength seawater in lagoons and bays, and a salinity of 35 is within their optimal growth range (Adams and Bate, 1994). With a field salinity range of some 2–40, *N. capensis* therefore provides an ideal subject for an examination of the extent to which intertidal seagrass faunal assemblages might vary along an estuarine axis. This paper then considers the effects on seagrass faunal biodiversity and related ecological features of the axial hydrographical gradient at the Knysna stronghold of *N. capensis*. The Knysna system is also an appropriate one for such an investigation on several grounds. It is, for example, the type of estuary in which salinity is likely to be an important factor structuring the fauna (Teske and Wooldridge, 2003), and its influence on biodiversity in several habitat types there has already been demonstrated (Day et al., 1952; Allanson et al., 2000a). Further, both *N. capensis* itself (Short et al., 2007, 2011) and the fauna that it supports at Knysna are of very high conservation importance (Hodgson and Allanson, 2000; Russell et al., 2009), and information on the spatial distribution of their biodiversity is of practical value to management strategy.

2. Study area

The permanently open, 18 km² Knysna estuarine bay (34002'S, 23001'E) is ranked ecologically as the most important of South Africa's 250+ estuaries (Turpie et al., 2002; Turpie and Clark, 2007). It contains a higher proportion of the total South African estuarine biodiversity than any other estuary (Turpie et al., 2002) and hosts a number of species for which Knysna is the only known African locality (Barnes, 2010; Barnes and Ellwood, 2011). In part this is due to its large size and wide range of habitat types, and also because, unusually amongst South African estuaries, it receives both seawater input with every high tide, supporting a diverse assemblage of marine species, and freshwater from a permanently inflowing river at its head. At the same time, however, it is also the most economically valuable of South Africa's estuarine resources. In 2007, for example, it was the premier estuarine tourist destination (with an annual tourist revenue then equivalent to 150×10^6 US\$) and premier estuarine residential area (with estuary-related property $\approx 0.2 \times 10^9$ US\$), whilst still yielding an annual income $\approx 26 \times 10^6$ US\$ from its use by line fish as a nursery-area and 120×10^3 US\$ of harvested resources to subsistence fishers (Turpie and Clark, 2007). However, despite the considerable human impingement on the system (Russell et al., 2009), levels of environmental health are still ranked as ‘good’, the second-highest category on a four point, ‘excellent’ → ‘poor’ scale (Turpie, 2004; Turpie and Clark, 2007).

Hydrographically, the estuarine bay is divisible into a linear series of three units (Largier et al., 2000): (1) an outer marine system tidally flushed with cool water from the Indian Ocean (the spring tidal prism being some 19×10^6 m³, the largest of any South African estuary; CSIR, 1974) and with salinities usually >34 ; (2) a middle, relatively isolated lagoonal section with not only a long residence time but also high salinity (30–34); and (3) an inner, stratified and well-flushed estuary with low ($0 < 30$) and variable salinity as a result of the influence of the Knysna River. Several other freshwater streams and small rivers do enter all three units, but these do not normally affect the hydrography of the main water mass. Estuaries being dynamic systems, these hydrographic components are not constant in areal extent or geographical position. The estuarine regime shrinks and enlarges in response to variation in river flow, and the marine regime likewise expands (to 7.5 km upstream) and contracts (to 4 km upstream) in response to variation in high-

tidal seawater incursion during spring (1.7 m range) versus neap (0.5 m range) tidal phases, forcing the intervening lagoonal regime to move accordingly (Largier et al., 2000). Further, the semi-diurnal tides impose short-term changes: during high tide the road and rail crossings (see Fig. 1) generally mark the approximate regions of transition from one hydrographic regime to the next; at low tide, all three regimes move seaward. The causeway-plus-bridge systems of these two crossings also effectively divide the estuarine bay into inner, middle and outer basins that thus, at high tide, roughly correspond to the three components of the hydrographic system. A representative salinity gradient along the estuary is shown in Fig. 2.

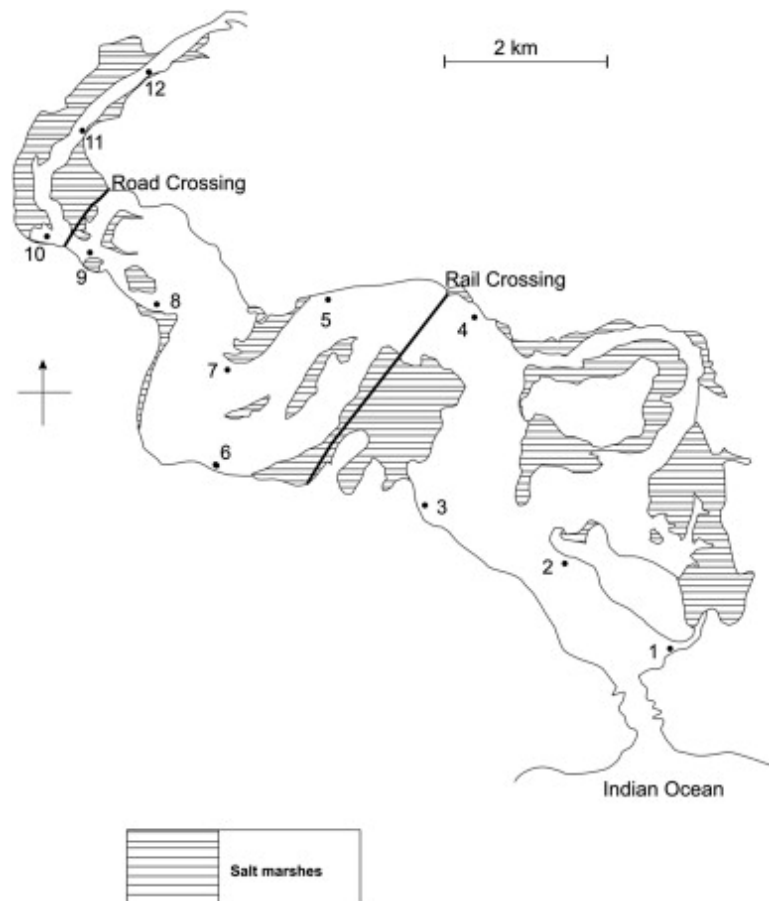


Fig. 1.

The Knysna estuarine bay showing the location of the 12 sites close to the margins of the axial channel. The width of the axial seagrass beds is at most sites too narrow for them to be included on a map of this scale.

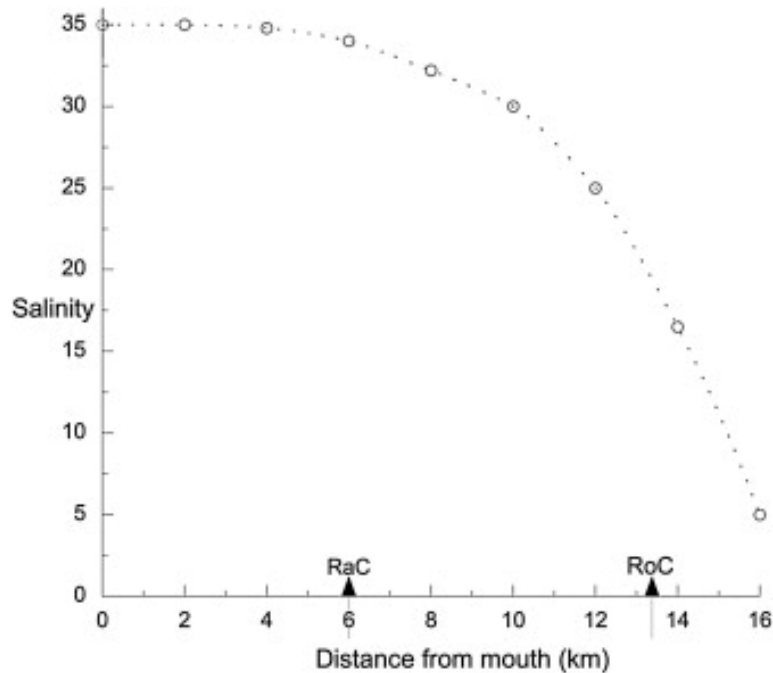


Fig. 2.

A representative gradient of surface water salinity at low tide along that section of the Knysna estuarine system supporting *Nanozostera capensis* (from data in Day et al., 1952; Hodgson and Allanson, 2000; and others). Distances along the X axis are as measured along the axial channel; RaC = rail crossing; RoC = road crossing.

Saltmarsh, seagrass and intertidal sediment flats occupy over half the area of the system (Maree, 2000; CES, 2009), the seagrass being overwhelmingly *N. capensis*, although *Halophila ovalis* also occurs throughout and *Ruppia maritima* replaces eelgrass in the upper reaches of the inner basin. In sheltered areas across the outer basin, *N. capensis* can occupy an intertidal height range of >1 m, cover horizontal distances of >400 m, and extend down to c. 1 m below MLWS (Grindley, 1985). Along the margins of the axial channel, however, eelgrass beds usually extend over much smaller horizontal and vertical distances, in the inner basin only over a vertical intertidal height of <0.3 m (Day, 1967; Maree, 2000), notwithstanding that the tidal range remains effectively unchanged (Grindley, 1985; Largier et al., 2000). Sediments vary somewhat within the estuary, clearly being less muddy near the mouth than elsewhere, as is typically the case in South African estuaries (Teske and Wooldridge, 2003), but otherwise the seagrass substratum ranges from firm sandy mud to softer sand-less mud in all three basins.

3. Methods

3.1. Sample collection and processing

To investigate the macrobenthic invertebrate assemblages of the intertidal seagrass along the estuary, sampling was conducted at 12 sites aligned in a chain over the 15.5 km of the axial channel that supports *N. capensis* (Fig. 1). Four such sites were located in each of the three basins, including one in each transitional hydrographic zone, and they were situated close to the axial channel to avoid potentially confounding variables such as the high degree of shelter present in the creeks between and behind islands (Barnes, 2010; Barnes and Ellwood, 2011). Three replicate stations, some 100 m apart, were worked at each site, and 10 core samples were taken 1 m apart in a line along the shore at each station. All cores were taken within the same shore height horizon, i.e. near the waterline during low water of spring tides <0.4 m in height. In the outer basin, this horizon corresponds to the lower quarter of the intertidal seagrass profile; in the inner basin, however, the horizon could virtually correspond to the bed itself (see above). Except at site 1 where the seagrass solely occurred in large patches, sampling stations were selected so that cores could be taken from within comparable areas of 100% cover in continuous seagrass beds to avoid confounding variables such as % ground cover, patch size and proximity to edges (Irlandi, 1994; Bowden et al., 2001; Tanner, 2005). Each of the 360 samples was of 27 cm² area and 50 mm depth, earlier studies in the Knysna system (Barnes,

2010) having indicated that 10 cores of that area would yield an acceptable standard error <10% of the arithmetic mean in estimation of animal numbers. A 50 mm depth was selected because most benthic macrofauna in seagrass beds occurs in the top few mm of sediment (e.g. 98% in the top 5 mm in the study by Klumpp and Kwak, 2005). Potential area effects on species richness and other parameters were avoided because the same total area was cored at each site and station. Sampling was carried out over a total period of three months during the 2011/2012 austral summer.

Treatment of the samples was the same as in earlier studies of spatial variation within the Knysna seagrass beds (Barnes, 2010; Barnes and Ellwood, 2011). Samples were collected soon after tidal ebb from the area of shore concerned, and were gently sieved through 710 μm mesh. Retained material was: (1) placed in a large polythene bag of seawater within which all seagrass fragments were shaken vigorously to dislodge all but sessile animals and then discarded; (2) it was then re-sieved and transported immediately to a local laboratory; where (3) it was placed in a large white tray from which the living fauna was extracted by eye, extraction continuing until no further animal could be seen after a 3 min search. Faunal individuals were identified whenever possible to species and were counted, excluding any sessile or semi-sessile animals (e.g. *Halianthella annularis*) that had become detached from the seagrass leaves during sorting. Occurrences at sites and stations are only as observed in the samples; i.e. without interpolation. Animal nomenclature is as listed in the World Register of Marine Species (WoRMS <www.marinespecies.org>), except for the unlisted *Rissoa capensis* Sowerby (on the basis of its anatomical and molecular taxonomy, this species is neither a *Rissoa* nor even a rissoid but is related to the Assimineidae; W.F. Ponder, pers. comm.: it is here referred to as 'R.' *capensis*). Across the inner basin a recent spatfall of the estuarine mussel *Arcuatula capensis* had occurred, and >8000 m^{-2} <2 mm long highly mobile young mussels crawling all over the *Nanozostera* leaves overwhelming dominated the fauna there, especially at two of the site 10 stations: this spatfall was excluded from calculations of species diversity, overall abundance, evenness and dominance.

3.2. Statistical analyses

Faunal assemblages were compared multivariately via hierarchical clustering analysis using S17 Bray–Curtis similarity, ordination by non-metric multidimensional scaling (nMDS), similarity percentage analysis (SIMPER), permutational analysis of multivariate dispersions (PERMDISP), including as a test for variability in between-group species composition (i.e. in β -diversity) (Anderson et al., 2006) and permutational analysis of variance (PERMANOVA), all carried out using PRIMER 6 [PrimerE Ltd: Plymouth Routines in Multivariate Ecological Research, Version 6]. Detrended correspondence analysis of the faunal assemblage data revealed a maximum gradient length >4 standard deviations, thus confirming that the data show a strong unimodal response around some environmental optima. Canonical correspondence analysis (CCA), via CANOCO for Windows 4.5 [Microcomputer Power, Ithaca NY], was therefore used to determine the proportion of assemblage variation attributable to the axial estuarine gradient. PERMANOVA, PERMDISP, SIMPER and nMDS were conducted on Bray–Curtis similarity matrices with fourth-root transformed versions of the data, PERMANOVA and PERMDISP using 9999 permutations.

Observed patterns of species co-occurrence were compared with statistical randomisations of the original species occurrence data using ECOSIM simulations (Gotelli and Entsminger, 2010). ECOSIM's default randomisation algorithm maintains fixed sums for row and column totals so that each matrix generated had the same number of species and core samples as the original. This algorithm has a low chance of falsely rejecting the null hypothesis (Type 1 error), but has good power for detecting non-random patterns (Gotelli, 2000). Differences between simulated and observed co-occurrence patterns were tested using the Stone and Roberts (1990) checkerboard score (C^-) index which indicates: random assemblage structuring if C_{obs} is not significantly different from C_{sim} ; non-random structure by virtue of more than expected negative co-occurrences if C_{obs} is significantly larger than C_{sim} ; and non-randomness by virtue of excess positive co-occurrences if C_{obs} is significantly smaller than C_{sim} .

Comparison of emergent assemblage properties at the various sites, i.e. species richness, α -diversity, evenness, dominance, and total abundance, used one-way ANOVA, followed by post-hoc Tukey HSD tests where applicable, and tests of potential correlation of these emergent properties with location along the axial gradient used the Pearson coefficient, in both respects after $\ln[x + 1]$ transformation. Otherwise tests of

potential correlation used the non-parametric Spearman rank coefficient. Granted the numerical importance of rare species in the local seagrass fauna (species represented by a single individual forming 26% of the 81 species found by Barnes, 2010 in a sample of 8644 individuals), observed numbers of species per site (Sobs) were converted to total species richness (Smax) by Chao-1 estimation (Chao, 1984). Species diversity (D) was measured as the linear Hill's (1973)N2 or the 'effective number of species' to permit quantitative comparison (Jost, 2006, 2007) and evenness then as Hill's (1973)N1/N0 (where N0 = Sobs); dominance was assessed by the Berger and Parker (1970)d as modified by Barnes and Barnes (2012) i.e. [(nmax – nmean)/N] where nmax is number of the most numerous species in a sample, nmean is mean number of individuals per species, and N is the total number. Patchiness was assessed via Morisita's Index of Dispersion and associated χ^2 analysis.

4. Results

4.1. General nature of the seagrass faunal assemblages

The survey yielded 100 species in total, all from essentially marine taxa except for the larvae of a chironomid midge, with a range of 12–50 (mean 29) species per site and with 25% of species represented by a single individual. The Chao-1 estimated total species richness was 135. Mean overall site densities ranged from 7918 to 3108 (mean 5364) ind. m⁻², and site α -diversities from 12.0 to 2.5 (mean 7.1). Species richness, α -diversity and overall density were all unevenly distributed through space (one-way ANOVA F11,24 > 7.4; P << 0.0001), although there was neither significant variation in values of evenness along the estuary (ANOVA F11,24 = 1.7; P = 0.14) (mean E per station 0.48, SE 0.01), nor in those of dominance (ANOVA F9,20 = 1.5; P = 0.22) (mean d per station 0.25, SE 0.01) until the upstream sites 11 and 12 at which levels of d increased to 0.47, SE 0.02. Even the species with the highest constancy index, *Nassarius kraussianus*, was very patchily distributed across all spatial scales from cores (Morisita's Id 1.86; χ^2 988; df 359; P << 0.0001) to sites (Id 1.22; χ^2 173; df 11; P << 0.0001).

Estimation of the components of spatial variance at the three nested sampling scales (PERMANOVA) showed that the component due to site was relatively large (c.f. Barnes, 2010; Barnes and Ellwood, 2011) and of a very similar magnitude to that at the level of the individual core samples (Table 1), indicating the importance of location along the estuary. As might be expected, faunal assemblages showed significant differentiation across the 12 sites (PERMANOVA pseudo-F = 25.9; P = 0.0001), with position along the axial gradient accounting for 29% of the total variation (CCA; eigenvalue 0.59; species–environment correlation = 0.97; Monte-Carlo randomisation test, P = 0.001), although pairwise PERMANOVA disclosed no significant differences between the assemblages at any given pair of sites (P ≥ 0.1). Non-metric multidimensional scaling (Fig. 3) shows that whilst site 2 formed an outlier (mainly consequent on the abundance there of *Turritella capensis* and of the unidentified ostracod; SIMPER) (see Table 2), the other six downstream sites (1 and 3–7) and the upstream five (8–12) formed separate clusters.

Table 1.

Partitioning of variance components at the nested spatial scales of site, station and core (PERMANOVA of fourth-root transformed data).

Spatial scale	df	Pseudo-F	P	\sqrt{EV}	VC
Site	11	9.19	0.0001	36.2	39.0
Station	24	3.26	0.0001	18.2	19.6
Core (=residual)	324			38.4	41.4
Total	359				

EV = estimate of variance; VC = variance component as percentage of the total.

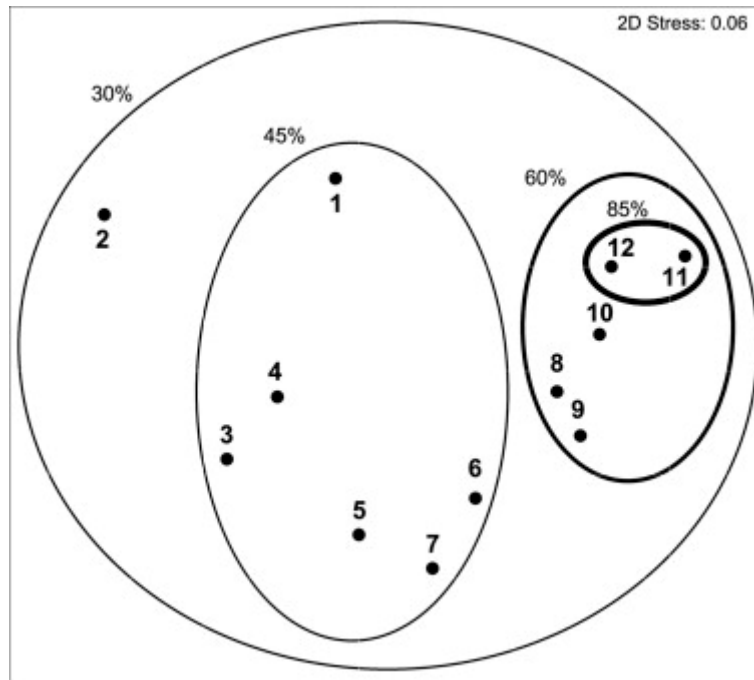


Fig. 3.

Non-metric multidimensional scaling plot of the superimposed group-average S17 Bray–Curtis Similarity clusters of fourth-root transformed abundances of the faunal assemblages at the 12 seagrass sites along the Knysna estuarine system. Envelope percentages stated are Bray–Curtis similarities.

Table 2.

Numerically-dominant members of the faunal assemblages at each of the twelve sites along the Knysna estuarine system (i.e. species comprising at least 7% of individuals at a site).

Site	1	2	3	4	5	6	7	8	9	10	11	12
<i>Symsagittifera macnaei</i>	–	–	–	–	10	–	8	–	–	–	–	–
<i>Ceratonereis erythraeensis</i>	–	8	23	15	–	16	–	17	–	22	–	–
<i>Capitella</i> sp.	26	–	–	–	–	–	–	–	–	–	–	–
<i>Prionospio sexoculata</i>	8	–	–	–	15	30	19	30	7	–	–	–
<i>Caulleriella capensis</i>	–	–	9	16	–	–	–	–	–	–	–	–
<i>Cirriformia</i> sp.	–	–	–	14	–	–	–	–	–	–	–	–
<i>Desdemona ornata</i>	–	–	11	–	–	–	–	–	–	–	–	–
Unidentified ostracod sp.	–	20	–	–	–	–	–	–	–	–	–	–
<i>Exosphaeroma hylecoetes</i>	–	–	13	–	–	–	–	–	–	–	–	–
<i>Diogenes brevirostris</i>	8	–	–	–	–	–	–	–	–	–	–	–
<i>Hydrobia knysnaensis</i>	–	–	–	–	–	–	–	–	–	–	60	52
' <i>Rissoa</i> ' <i>capensis</i>	–	–	–	–	–	–	–	–	–	–	7	12
<i>Turritella capensis</i>	–	20	–	–	–	–	–	–	–	–	–	–
<i>Nassarius kraussianus</i>	22	–	7	–	18	12	23	13	42	27	10	10
<i>Arcuatula capensis</i>	–	–	–	7	–	–	7	27	29	28	14	14

Dashes indicate that a species comprised <7% of numbers at a site, not necessarily that it was absent.

Comparisons of observed and simulated species co-occurrence patterns showed no differentiation in the organisation of the seven downstream and the five upstream sites. At the spatial scale of site, a similar proportion (five of the seven downstream and four of the five upstream sites) did not depart from random, whilst at the larger spatial scale of the upstream versus downstream site-groups both were deterministically structured ($C_{obs} > C_{sim}$; $P = 0.01$ and 0.03). However, in respect of the five upstream sites, this result was entirely dependent on *Prionospio* and *Hydrobia*; with either of these species removed from the data set, structure of the remaining assemblage did not then depart from random ($C_{obs} > C_{sim}$; $P > 0.3$). Deletion of any of the other three dominants (*Nassarius*, *Ceratonereis* or *Arcuatula*), in contrast, had no such effect.

4.2. Downstream sites (1–7)

Species richness per station was not uniform between sites 1 and 7 (one-way ANOVA $F_{6,14} = 6.3$; $P < 0.005$), consequent on significant differences between site 6 and sites 2, 4 and 5 (Tukey $P < 0.015$), and neither was assemblage composition (PERMANOVA pseudo- $F = 6.1$; $P = 0.0001$) (Table 2). However, there was no significant correlation between position along the upstream axis and either the observed or the estimated total number of species per station or per site over this region (Pearson $P > 0.2$) (Fig. 4A). Neither was there any significant variation in α -diversity (Fig. 4B) (ANOVA $F_{6,14} = 0.9$; $P = 0.5$), nor any correlation of α -diversity with position along the axis (Pearson $P = 0.8$). On average, each of the seven downstream sites supported 40% (SE 2.9) of the local pool of 97 observed species, of which 24% were represented by singletons and a further 10% by doubletons, and each supported 41% (SE 4.3) of the estimated local total of 123 species. Correspondingly, levels of within-site Bray–Curtis similarity were low, averaging 35.6% SE1.5 (one-way SIMPER). Within this downstream region's diverse fauna only three species (*N. kraussianus*, *Ceratonereis erythraeensis* and *Prionospio sexoculata*) attained $>7\%$ of total numbers or occurred in $>33\%$ of samples; together they accounted for $<32\%$ of total individuals (Fig. 5). As expected, position along the axis accounted for a relatively small, though still significant, proportion (32.8%) of the assemblage variation among these downstream sites (CCA; eigenvalue 0.16; species–environment correlation = 0.89; Monte-Carlo randomisation test, $P = 0.002$).

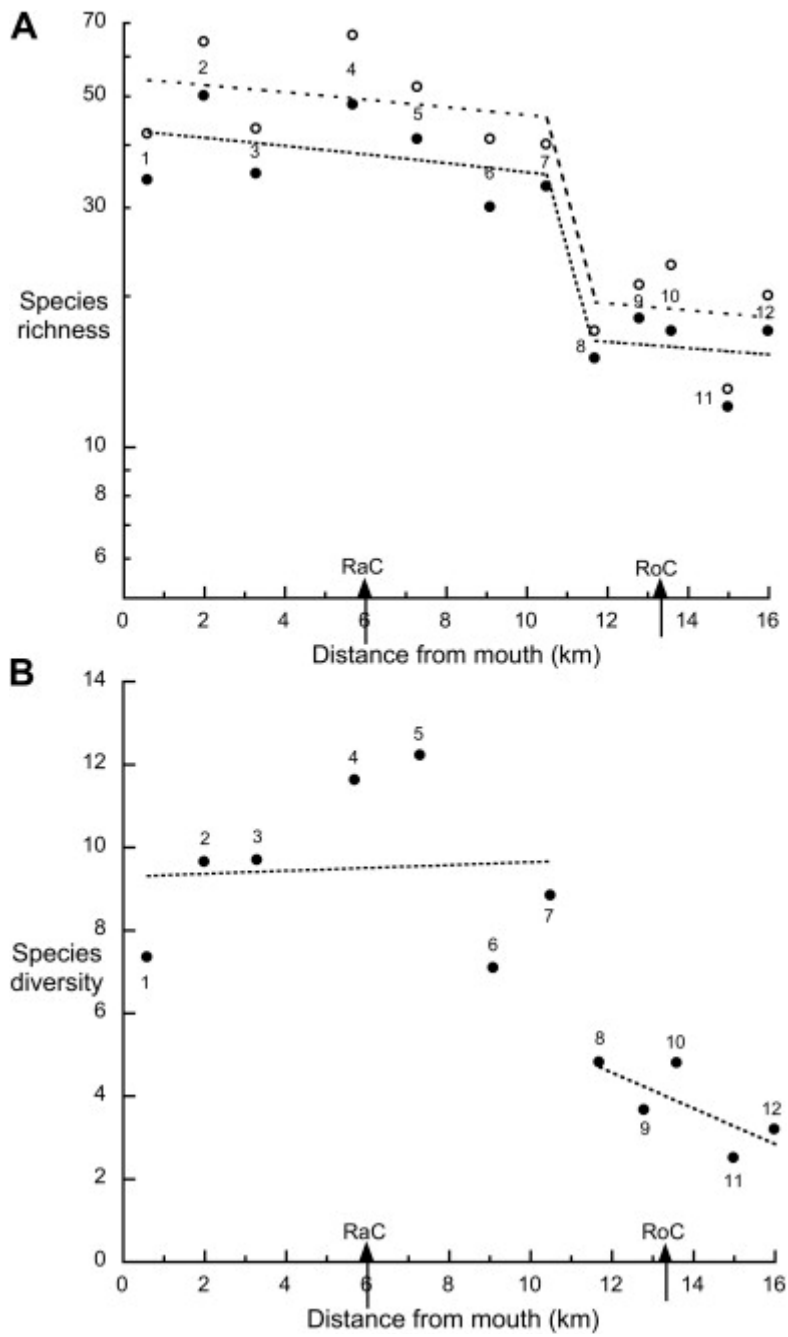


Fig. 4.

Macrobenthic faunal biodiversity within intertidal seagrass at sites 1–12 along the length of the Knysna estuarine system. A; Observed (●) and Chao-1 estimated total (○) numbers of species per site. Note the sharp fall at some 11 km upstream; variation within each of the two separated plateaux is not statistically significant. B; α -species diversity (effective number of species) per site. For details of the X axis see Fig. 2.

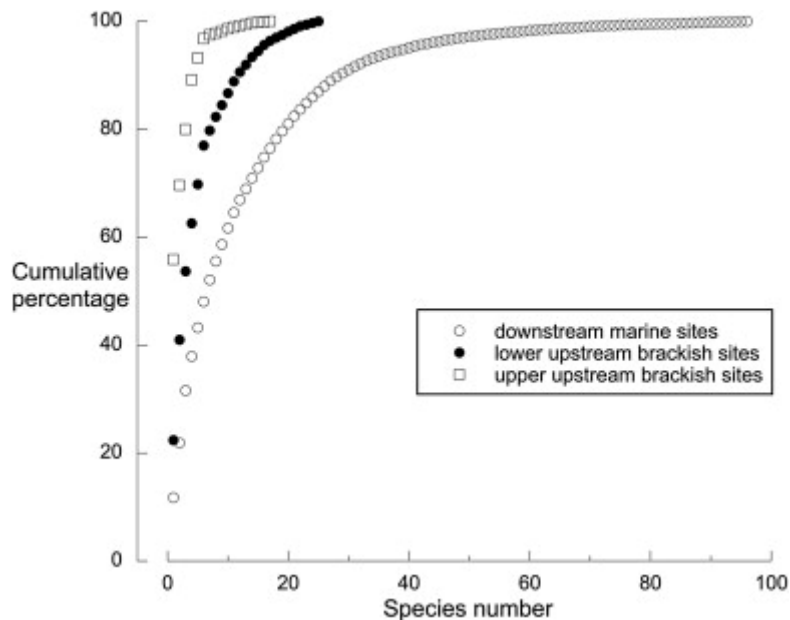


Fig. 5.

Differing faunal assemblage structure in different regions of the Knysna estuarine system. Cumulative relative abundance, as percentage of total individuals, of the macrobenthic species within intertidal seagrass in the downstream high-salinity sites (1–7), lower upstream brackish sites (8–10) and upper upstream brackish sites ('upstream *Hydrobia* zone') (11 and 12).

4.3. Upstream sites (8–12)

A marked decrease in species richness, however, was evident upstream of site 7 (Fig. 4A); site 8 differing significantly in this respect from all seven downstream sites (Tukey $P < 0.006$ in each case). Likewise site 8 showed a significant difference in β -diversity from each of the downstream sites (pairwise PERMDISP $t > 2.5$; $P < 0.02$). This lower level of richness was then maintained without any statistically significant variation (ANOVA $F_{4,10} = 1.3$; $P = 0.3$) or correlation with distance upstream (Pearson $P = 0.8$) across all the five upstream sites (Fig. 4A). There was, however, a significant variation in α -diversity upstream from site 7 (ANOVA $F_{4,10} = 5.3$; $P = 0.01$), although solely consequent on the difference between sites 8 and 11 (Tukey $P = 0.01$), and there was a significant negative correlation between diversity and distance upstream (Pearson $RP = -0.67$; $P < 0.01$) (Fig. 4B). In the latter case this was consequent on a marked change in assemblage composition occurring between sites 10 and 11 (see below). Each of the five sites upstream of site 7 supported a much greater percentage, an average 57% (SE 3.8), of the much smaller local pool of 28 observed species (estimated total 32). 18% of the upstream fauna were represented by singletons and a further 11% by doubletons, without significant change from the downstream sites in proportion of such rarities per site (ANOVA $F_{1,10} > 1.8$; $P > 0.1$). Densities of *Ceratonereis* and *Prionospio* did not differ between the two stretches of the estuary (ANOVA $F_{1,34} < 0.7$; $P > 0.4$) although *Arcuatula* was much more abundant upstream than downstream (ANOVA $F_{1,34} = 114$; $P \ll 0.0001$). Only three species were present upstream of site 7 that were not also obtained downstream, and all three are known to be part of the downstream fauna as well, having been recorded there by the surveys of Allanson et al. (2000a) and/or Barnes (2010). The upstream fauna was then a small subset of the richer downstream assemblage, not a separate brackish-water association of replacement species. Unusually for an echinoderm, the upstream subset included the burrowing holothurian *Leptosynapta knysnaensis* which thus penetrates further upstream, and into lower salinities, than previously recorded (e.g. by Day et al., 1952). Location along the upstream axis accounted for a higher proportion (54.2%) of the assemblage variation among sites 8–12 (CCA; eigenvalue, 0.07; species–environment correlation, 0.92; Monte-Carlo randomisation test, $P = 0.002$) than it did among the downstream marine-influenced ones.

The upstream sites were themselves divisible into two groups in that a second significant faunal discontinuity in β -diversity (pairwise PERMDISP $t > 4.5$; $P < 0.0001$), though not in species richness

(see above), occurred within the upstream zone between sites 10 and 11. Across the lower of the two upstream zones (sites 8–10), the same three dominant species as downstream, together with *A. capensis*, were again the only ones to attain >7% of total numbers or occur in >33% of samples, but together they here comprised a much higher percentage (>82%) of the fauna (see Fig. 5) (c.f. sites 1–7 above). This was reflected in higher levels of within-site similarity than downstream (mean 51.3% SE 0.9; one-way SIMPER), with most of the similarity being attributed to a total of only three species. Assemblages did not differ significantly across these three sites (PERMANOVA pseudo-F = 2.9; $P > 0.08$). Although *Nassarius*, *Ceratonereis* and *Arcuatula* remained as abundant upstream of site 10 as over the lower upstream zone of sites 8–10 (ANOVA $F_{1,13} < 0.9$; $P > 0.3$), their numbers were dwarfed at sites 11 and 12 by the additional presence of very high densities of *Hydrobia knysnaensis*, and to a lesser extent '*R.*' *capensis*, which were responsible for 41% of the dissimilarity between sites 10 and 11 (one-way SIMPER). Sites 1–10 supported an average of only <3 *Hydrobia* and <9 '*Rissoa*' m^{-2} , whereas at site 11 the position changed dramatically to >4500 *Hydrobia* and >500 '*Rissoa*' m^{-2} and the two comprised 67% of the faunal individuals there. This microgastropod domination continued at site 12. Within-site Bray–Curtis similarities were highest at sites 11 and 12 (mean 70.7% SE 1.0; one-way SIMPER), levels of within-site similarity differing significantly between the three groups of sites along the estuary (i.e. sites 1–7, 8–10 and 11–12) (ANOVA $F_{2,9} = 87$; $P < 0.0001$). Species richness at sites 11 and 12 did not differ from those at sites 8–10 (ANOVA $F_{1,13} < 0.01$; $P = 0.9$), but their species diversities did so (ANOVA $F_{1,13} = 12.5$; $P < 0.01$).

4.4. Gradual changes in assemblage features

Besides the stepped changes occurring between sites 7 and 8 and between sites 10 and 11 indicated above, there were also indications of gradual upstream trends in faunal assemblages and in their characteristics. The nMDS plot (Fig. 3), for example, also shows that there was a significant trend for the site assemblages to distribute themselves across the Euclidean space in their order up the estuary (Spearman $RS > 0.9$; $P < 0.0001$). Secondly, overall faunal abundance was not uniform along the estuary (ANOVA $F_{11,24} = 7.3$; $P < 0.0001$) (Fig. 6), and it also decreased significantly with distance upstream until the upstream *Hydrobia* zone (Pearson $RP = -0.69$; $P < 0.001$). Over the same stretch of estuary there was a strong positive correlation between faunal density and observed species richness (Pearson $RS = 0.6$; $P < 0.001$). Of the 21 most widespread species between sites 1 and 10 (those occurring in at least 10% of samples), however, the abundance of 15 of them did not correlate with distance upstream (Spearman RS ; $P > 0.05$) and the density of three of them — *Arcuatula* (see above), *Glycera convoluta* and *Macoma litoralis* — actually increased significantly (Spearman $RS > 0.7$; $P < 0.04$). Only densities of the polychaetes *Arabella iricolor*, *Orbinia angrapequensis* and *Aricidea capensis* decreased significantly with upstream distance (Spearman $RS > 0.7$; $P < 0.02$). During this decline in overall density, the proportion of polychaetes remained unaffected (Spearman $RS = -0.37$; $P = 0.3$) but those of crustaceans fell (Spearman $RS = -0.83$; $P = 0.003$) and of molluscs increased (Spearman $RS = 0.68$; $P = 0.03$). At the two most upstream sites (11 and 12) faunal abundance rose sharply again (Fig. 6), molluscs here comprising >93% of the individuals.

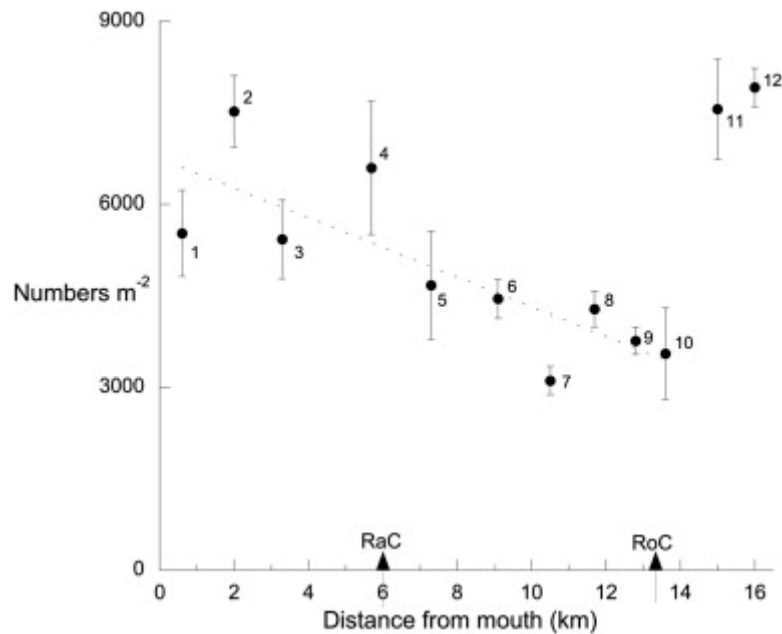


Fig. 6.

Overall abundance of macrobenthic fauna (mean numbers $m^{-2} \pm 1$ standard error at each site) within intertidal seagrass at sites 1–12 along the length of the Knysna estuarine system. For details of the X axis see Fig. 2.

5. Discussion

Salinity gradients of tidal estuaries such as that at Knysna are far from static. In times of flood (that occur once every 10–12 years; Day et al., 1952) during which river flow may reach $170 \text{ m}^3 \text{ s}^{-1}$ (Largier et al., 2000), freshwater conditions can occur throughout all three basins, especially during low tide. The last such occasion was some 18 months before the present samples were taken (B.R. Allanson, pers. comm.). During droughts, river flow can decrease to such a low level ($<0.4 \text{ m}^3 \text{ s}^{-1}$) that it only balances evaporation and evapotranspiration and no net seaward flow occurs; hence salinities of >30 can extend right through the system especially during high tide (Largier et al., 2000). Therefore over the medium term, all intertidal points throughout the entire estuarine bay may experience a maximum of >30 and a minimum of <5 , and patterns of distribution in some estuaries, especially in otherwise high-salinity regions, can be driven by such extreme events (Jones, 1987). Under non-extreme conditions, however, Largier et al. (2000) suggest that all seagrass regions in the outer and middle basins at the height sampled are likely to be bathed by water >30 ; whereas stratification within the inner basin is likely to produce wide semi-diurnal salinity fluctuation with state of the tide, from highs of >28 to lows of: down to 20 at site 9; 8 at site 10; 5 at site 11; and <3 at site 12 (Maree, 2000; Allanson et al., 2000b). If then the general axial salinity gradient (whether its mean values or the extent of short-term variation) is a major determinant of seagrass faunal biodiversity and related ecological features, and if the model of Largier et al. (2000) adequately describes the salinity regime, three predictions follow: (1): that faunal biodiversity, composition, structure, etc. should (all other things being equal) remain unchanged within the 10.5 km zone of >30 salinity (from Site 1 to Site 8), as is the case, for example, in the seawater-dominated Kariega Estuary in the Eastern Cape (Henninger and Froneman, 2011); (2): that the critical point along the length of the Knysna estuarine system should be the junction between the middle and inner basins, i.e. the general vicinity of Site 9; (3): that the rapidly decreasing minimum salinities upstream of that critical point, at least during low tide, should result in declining biodiversity thereafter.

Such predictions might not seem to be supported by the Knysna fauna as a whole, in that a relatively even, continual reduction in species richness per site has been recorded (Day et al., 1952, as updated by Day, 1967), from >175 near the mouth to <35 near the head, although the same trend has not been seen in (Shannon) species diversity (Allanson et al., 2000a). These data, however, were derived from all habitat types and from all tidal heights at each sampling point, and both habitat and tidal height

can be powerful confounding variables (Day, 1967; Teske and Wooldridge, 2003). Both habitat diversity and hard substrata decrease upstream, for example, potentially obscuring any potential relationship between species richness and salinity.

In contrast, the present study was restricted to a single habitat type and within that to a single tidal height. These data showed that faunal richness within the *N. capensis* did essentially conform to two of the local salinity-regime based predictions. Richness across the seven downstream sites along the axial channel was high, Sobs per site averaging 39 and Smax 50, and, although variable from site to site as it is across the entire outer basin (Barnes, 2010), it did not vary with distance from the mouth until a point some 11 km upstream after which there was a sharp decrease to Sobs < 19 and Smax < 24. Although this critical zone between sites 7 and 8 is somewhat further downstream than might be predicted, both Day et al. (1952) and Allanson et al. (2000b) do show that average salinity in the vicinity of site 8 drops to 25 at low tide, and the sudden decrease in species richness therefore does correspond to the region of the Knysna estuarine bay in which salinities typically drop below 30 for part of each tidal cycle.

Biodiversity at the five upstream sites did not conform to the third of the three predictions, however. Rather than decline with distance upstream, species richness showed no significant variation but plateaued at a level some 40% of that in the marine-dominated region, Sobs per site averaging 16 and Smax 19, along a section of the estuary over which minimum salinity decreased from 25 to <3 and its range increased from 10 to >25, i.e. well into the zone generally regarded as being critical for estuarine biodiversity (Telesh and Khlebovich, 2010; Elliott and Whitfield, 2011; Whitfield et al., 2012). Although species richness per station and site were thus still relatively high, the total upstream fauna was nevertheless impoverished in relation to that downstream: the local pool of species was <30% of that in the marine section (although note that the area sampled was only 70% of the latter) and just five species comprised >83% of the total numbers of individuals, whereas the same five comprised <35% of the downstream total. The presence in large numbers of the microgastropods *H. knysnaensis* and '*R.*' *capensis* only at the two most upstream sites, at which salinities can fall below 3 during low tide, might suggest that salinity has a strong influence on their distribution and that they are brackish-water specialists. This, however, is not the case; indeed, like its closest relative *Hydrobia ulvae* (Hylleberg, 1986; Pascual and Drake, 2008; etc.), *H. knysnaensis* appears biologically to be an essentially euhaline species also capable of withstanding dilute media (Barnes, 2004). Abundance of *H. knysnaensis* and '*R.*' *capensis*, both local endemics restricted to the Western Cape and the western part of the Eastern Cape, shows a peculiar, markedly disjunct distribution in the Knysna system. Besides dominating the lowest salinity reaches of the estuary at low tidal levels (Barnes, 2002; and present study), the two also dominate the seagrass fauna at relatively high-tidal levels in fully marine areas of the Knysna outer basin near the mouth of the system, where, in 2010, mean densities of 16,000 ind. m⁻² occurred in *N. capensis* beds at the head of very sheltered backwater creeks enclosed within blocks of saltmarsh (Barnes, 2010). They are, however, scarce to absent along the exposed axial channel in the outer basin, through the intervening middle basin and in the downstream section of the inner basin (Barnes, 2004, 2010; and present study). The two regions that they can at times dominate are both extreme, having the lowest salinities or the highest tidal levels tolerated by seagrass respectively. The microgastropods could thus be considered marginal species occupying the fringes of the habitat range of seagrass within the estuary, albeit at times very successfully although their numbers fluctuate markedly from year to year (Barnes, 2004; Barnes and Ellwood, 2011). There are no indications of their occurrence only in areas from which some potential competitor is absent, so the driver may be escape from predation by fish (such as grunters, Pomadasys). In respect of competition, it was noticeable that except for these two microgastropods at sites 11 and 12, overall faunal densities declined markedly (halved) on progression upstream and those of only three species increased (two suspension feeding bivalves, a relatively insignificant feeding category downstream, and a predatory polychaete only present in small numbers anyway). There was, therefore, little evidence of any putative increase in abundance of the remaining species on release from competition.

The upstream and numerically-dominant downstream species at Knysna are classic members of the southern African estuarine fauna (Day, 1981; Schlacher and Wooldridge, 1996; de Villiers et al., 1999; Henninger and Froneman, 2011). Although characteristic of estuaries, they clearly occur in full-strength seawater in the Knysna outer basin and many can withstand salinities of up to 50–55 (de Villiers et al., 1999; Whitfield et al., 2012). That they are not commonly found in the sea appears more a matter of the absence of other suitable shallow, sheltered habitat along the South African coastline (Day, 1967; Cooper, 2001) than of the specifically ‘estuarine’ nature of the species themselves – the exception proving this rule being the fauna of the sheltered but non-estuarine Langebaan inlet which shows very close similarities to that at Knysna (see e.g. Hanekom et al., 2009). It might be argued that as this South African estuarine fauna is known to be relatively independent of ambient salinity (de Villiers et al., 1999; Teske and Wooldridge, 2001, 2003, 2004) it is only to be expected that salinity would not affect their upstream distributions, particularly as the stretches of the Knysna Estuary supporting seagrass do not include the oligohaline zone, and therefore that the constancy of species composition and richness across sites 8–12 has nothing to do with the seagrass habitat. Other evidence suggests that this is not so. Despite their independence of salinity, Schlacher and Wooldridge (1996) demonstrated that this fauna showed a decline in species richness over a range of minimum salinities from <30 to 10 in unvegetated sediments along the Gamtoos Estuary (Eastern Cape) and also that there they formed an axial series of distinct assemblages upstream. Distributions of the typically estuarine benthic species of northwestern Europe are also largely independent of salinity above a 4–5 lower limit (Barnes, 1989), yet in unvegetated brackish-water sediments they also show declines in species richness down gradients of the same range in minimum salinity (Bleich et al., 2011; Rodrigues et al., 2011; Whitfield et al., 2012; etc.).

It then appears that there is a considerable degree of macrobenthic assemblage stability in areas of the Knysna estuarine system subject to minimum salinities of from 25 to <3. In their potentially protective vegetated habitat, a sizable group of species, all (with the possible exception of *Arcuatula*) equally characteristic and indeed often dominant members of the fauna of various regions of the marine-influenced zone downstream, can also survive low and wildly fluctuating salinities upstream. Clearly, however, not all species do so. Day (1967, 1981) considered that 139 of the 310 marine invertebrates that he and his colleagues had recorded from the Knysna system were stenohaline marine in nature. The present survey disclosed that a pool of 97 observed (123 estimated total) species occurred at the marine sites 1–7, but that this dropped to 28 observed (32 estimated total) species at the upstream sites 8–12. Many of the species not present upstream were relatively rare components of the downstream fauna although some, such as *T. capensis*, *Paridotea unguolata*, *Desdemona ornata* and various eunicidan and scolecidan polychaetes, were common, characteristic downstream species, not only along the axial channel but also in more sheltered regions (Barnes, 2010; Barnes and Ellwood, 2011). Species such as these and the others responsible for the observed fall in richness are most likely to be mainly this stenohaline marine component (see Teske and Wooldridge, 2003). Apart from those, however, there was no indication of the restriction of particular species or assemblages to different salinity zones, the spurious case of *Hydrobia* and ‘*Rissoa*’ excepted. That the switch from the full range of downstream marine species to the more limited upstream group of estuarine species occurred over a very short stretch of the Knysna system — a maximum distance of 1.2 km — is unexpected and potentially very interesting. It suggests the possibility that within the seagrass the fauna shows not so much the more usual seaward ecocline (Attrill and Rundle, 2002) as a sharp ecotone, and further research to determine whether the boundary zone is unstable and dynamic could be potentially rewarding.

Finally, does the distribution of macrobenthic species richness within the Knysna estuarine seagrass meadows then conform to the Remane paradigm? Much would seem to depend on what features of the model are considered most critical. Leaving aside uncertainties associated with the most appropriate units of its salinity axis (in a tidal estuary should these be the absolute minimum, the mean, the range, etc?), all variants of the Remane diagram reviewed by Whitfield et al. (2012) posit a steep but gradual decrease in species richness between estuarine regions subject to salinities of from 30 to 15 (the approximate mean value at Knysna site 12) or <3 (the minimum at that site). In

contrast, at Knysna there was no significant decline in richness over this segment of the axis, suggesting independence of richness from salinity over this range. However, when considering the results of Jones et al. (1986) on the relationship between benthic species richness and salinity along the Australian Hawkesbury Estuary, Whitfield et al. (2012, p. 85) also considered that 'although a consistent decline in species richness along the system never occurred, the more saline locations usually did support the majority of species and the upstream locations had the least species (i.e. conforming to the Remane model)' (our italics), implying that in their view the essence of the model is species-poverty of the brackish stretch rather than decrease in richness with decreasing salinity within that stretch. The more saline sites 1–7 at Knysna each certainly supported greater species richness than did the upstream brackish sites 8–12 and therefore in those terms the estuary's seagrass fauna does conform.

Acknowledgements

RSKB is most grateful to the Rondevlei Offices of SANParks and the Knysna Area Manager, Andre Riley, for permission to undertake fieldwork in the Knysna Coastal Section of the Garden Route National Park; to Rhodes University Research Committee for financial support; to Karen Diedericks for producing Fig. 1; to Peter and Frances Smith for field assistance; and to Brian Allanson for his constant support. MDFE is grateful for the support of the University Museum of Zoology and the Department of Zoology, University of Cambridge.

References

- Adams, J.B. & Bate, G.C. 1994, "The ecological implications of tolerance to salinity by *Ruppia cirrhosa* (Petagna) Grande and *Zostera capensis* Setchell", *Botanica Marina*, vol. 37, no. 5, pp. 449-456.
- Alexander, W.B., Southgate, B.A. & Bassindale, R. 1935, *Survey of the River Tees.II.The Estuary - Chemical and Biological*, .
- Alexander, W.B., Southgate, B.A. & Bassindale, R. 1932, "The salinity of the water retained in the muddy foreshore of an estuary", *Journal of the Marine Biological Association*, vol. 18, pp. 297-298.
- Allanson, B.R., Maree, B. & Grange, N. 2000, "An introduction to the chemistry of the water column of the Knysna estuary with particular reference to nutrients and suspended solids", *Transactions of the Royal Society of South Africa*, vol. 55, pp. 141-162.
- Allanson, B.R., Nettleton, J. & De Villiers, C.J. 2000, "Benthic macrofauna richness and diversity in the Knysna Estuary: A 50 year comparison", *Trans.R.Soc.S.Afr.*, vol. 55, pp. 177-185.
- Anderson, M.J., Ellingsen, K.E. & McArdle, B.H. 2006, "Multivariate dispersion as a measure of beta diversity", *Ecology Letters*, vol. 9, no. 6, pp. 683-693.
- Attrill, M.J. 2002, "A testable linear model for diversity trends in estuaries", *Journal of Animal Ecology*, vol. 71, no. 2, pp. 262-269.
- Attrill, M.J. & Rundle, S.D. 2002, "Ecotone or ecocline: Ecological boundaries in estuaries", *Estuarine, Coastal and Shelf Science*, vol. 55, no. 6, pp. 929-936.
- Bandeira, S.O. & Gell, F. 2003, "The seagrasses of Mozambique and southeastern Africa", *World Atlas of Seagrasses*, , pp. 93-100.
- Barnes, R.S.K. 2010, "Spatial variation in abundance and diversity of the smaller surface and near-surface eelgrass-associated intertidal macrobenthos within a warm-temperate estuarine bay in the Garden Route National Park, RSA", *Aquatic Conservation: Marine and Freshwater Ecosystems*, vol. 20, no. 7, pp. 762-772.

- Barnes, R.S.K. 2004, "The distribution and habitat in the Knysna Estuary of the endemic South African mudsnail *Hydrobia knysnaensis* and the influence of intraspecific competition and ambient salinity on its abundance", *African Journal of Aquatic Science*, vol. 29, no. 2, pp. 205-211.
- Barnes, R.S.K. 2002, "The occurrence and ecology of a marine hydrobiid mudsnail in the southern hemisphere: The Knysna Estuary, South Africa", *African Journal of Ecology*, vol. 40, no. 3, pp. 289-294.
- Barnes, R.S.K. 1989, "What, if anything, is a brackish-water fauna?", *Transactions - Royal Society of Edinburgh: Earth Sciences*, vol. 80, no. 3-4, pp. 235-240.
- Barnes, R.S.K. & Barnes, M.K.S. 2012, "Shore height and differentials between macrobenthic assemblages in vegetated and unvegetated areas of an intertidal sandflat", *Estuarine, Coastal and Shelf Science*, vol. 106, pp. 112-120.
- Barnes, R.S.K. & Ellwood, M.D.F. 2011, "The significance of shore height in intertidal macrobenthic seagrass ecology and conservation", *Aquatic Conservation: Marine and Freshwater Ecosystems*, vol. 21, no. 7, pp. 614-624.
- Bassindale, R. 1943, "The physical environment and intertidal fauna of the southern shores of the Bristol Channel and Severn Estuary", *Journal of Ecology*, vol. 31, pp. 1-29.
- Berger, W.H. & Parker, F.L. 1970, "Diversity of planktonic foraminifera in deep-sea sediments", *Science*, vol. 168, no. 3937, pp. 1345-1347.
- Bleich, S., Powilleit, M., Seifert, T. & Graf, G. 2011, "β-diversity as a measure of species turnover along the salinity gradient in the baltic sea, and its consistency with the venice system", *Marine Ecology Progress Series*, vol. 436, pp. 101-118.
- Bowden, D.A., Rowden, A.A. & Attrill, M.J. 2001, "Effect of patch size and in-patch location on the infaunal macroinvertebrate assemblages of *Zostera marina* seagrass beds", *Journal of experimental marine biology and ecology*, vol. 259, no. 2, pp. 133-154.
- CES, 2009, *Knysna N2 Toll Highway, Final Environmental Impact Report, November 2009*
- Chao, A. 1984, "Non-parametric estimation of the number of classes in a population", *Scandinavian Journal of Statistics*, vol. 11, pp. 265-270.
- Cognetti, G. & Maltagliati, F. 2000, "Biodiversity and adaptive mechanisms in brackish water fauna", *Marine pollution bulletin*, vol. 40, no. 1, pp. 7-14.
- Collett, L.C., Hutchings, P.A., Gibbs, P.J. & Collins, A.J. 1984, "A comparative study of the macro-benthic fauna of *Posidonia australis* seagrass meadows in New South Wales", *Aquatic Botany*, vol. 18, no. 1-2, pp. 111-134.
- Cooper, J.A.G. 2001, "Geomorphological variability among microtidal estuaries from the wave-dominated South African coast", *Geomorphology*, vol. 40, no. 1-2, pp. 99-122.
- CSIR, 1974, *Proposed Braamekraal Marina, Knysna Model Studies*
- Day, J.H. 1981, *Estuarine Ecology with Particular Reference to Southern Africa*, .
- Day, J.H. 1967, "The biology of Knysna Estuary, South Africa", *Estuaries*, , pp. 397-407.

- Day, J.H., Millard, N.A.H. & Harrison, A.D. 1952, "The ecology of South African estuaries. Part III Knysna: A clear open estuary", *Transactions of the Royal Society of South Africa*, vol. 33, no. 3, pp. 367-413.
- De Biasi, A.M., Benedetti-Cecchi, L., Pacciardi, L., Maggi, E., Vaselli, S. & Bertocci, I. 2003, "Spatial heterogeneity in the distribution of plants and benthic invertebrates in the lagoon of Orbetello (Italy)", *Oceanologica Acta*, vol. 26, no. 1, pp. 39-46.
- De Villiers, C., Hodgson, A. & Forbes, A. 1999, "Studies on estuarine macroinvertebrates", *Estuaries of South Africa*, , pp. 167-208.
- Edgar, G.J. & Barrett, N.S. 2002, "Benthic macrofauna in Tasmanian estuaries: Scales of distribution and relationships with environmental variables", *Journal of experimental marine biology and ecology*, vol. 270, no. 1, pp. 1-24.
- Elliott, M. & Whitfield, A.K. 2011, "Challenging paradigms in estuarine ecology and management", *Estuarine, Coastal and Shelf Science*, vol. 94, no. 4, pp. 306-314.
- Gotelli, N.J. 2000, "Null model analysis of species co-occurrence patterns", *Ecology*, vol. 81, no. 9, pp. 2606-2621.
- Gotelli, N.J. & Entsminger, G.L. 2001, *EcoSim: Null Models Software for Ecology. Version 6.0*, .
- Grindley, J.R. 1985, *Estuaries of the Cape Part II. Synopses of Available Information on Individual Systems. Report No.30: Knysna*, .
- Hanekom, N., Randall, R.M., Nel, P. & Kruger, N. 2009, "West Coast National Park, State of Knowledge", *West Coast National Park. State of Knowledge*, .
- Henninger, T.O. & Froneman, P.W. 2011, "Macrofaunal community structure in the littoral zone of a freshwater-deprived, permanently open Eastern Cape Estuary", *African Zoology*, vol. 46, no. 2, pp. 263-279.
- Hill, M.O. 1973, "Diversity and evenness: A unifying notation and its consequences", *Ecology*, vol. 54, no. 2, pp. 427-432.
- Hodgson, A.N. & Allanson, B.R. 2000, "The Knysna Basin Project 1995-1998. A scientific report on the Knysna estuary", *Transactions Royal Society of South Africa*, vol. 55, pp. 95-240.
- Hylleberg, J. 1986, "Distribution of hydrobiid snails in relation to salinity, with emphasis on shell size and co-existence of the species", *Ophelia*, vol. 4, no. SUPPL., pp. 85-100.
- Irlandi, E.A. 1994, "Large- and small-scale effects of habitat structure on rates of predation: How percent coverage of seagrass affects rates of predation and siphon nipping on an infaunal bivalve", *Oecologia*, vol. 98, no. 2, pp. 176-183.
- Iyer, V. & Barnabas, A.D. 1993, "Effects of varying salinity on leaves of *Zostera capensis* Setchell. I. Ultrastructural changes", *Aquatic Botany*, vol. 46, no. 2, pp. 141-153.
- Jones, A.R. 1987, "Temporal patterns in the macrobenthic communities of the Hawkesbury estuary, New South Wales", *Australian Journal of Marine & Freshwater Research*, vol. 38, no. 5, pp. 607-624.
- Jones, A.R., Watson-Russell, C.J. & Murray, A. 1986, "Spatial patterns in the macrobenthic communities of the Hawkesbury Estuary, New South Wales.", *Australian Journal of Marine & Freshwater Research*, vol. 37, no. 4, pp. 521-543.

- Jost, L. 2007, "Partitioning diversity into independent alpha and beta components", *Ecology*, vol. 88, no. 10, pp. 2427-2439.
- Jost, L. 2006, "Entropy and diversity", *Oikos*, vol. 113, no. 2, pp. 363-375.
- Kinne, O. 1967, "Physiology of estuarine organisms with special reference to salinity and temperature: General aspects", *Estuaries*, , pp. 525-540.
- Klumpp, D.W. & Kwak, S.N. 2005, "Composition and abundance of benthic macrofauna of a tropical sea-grass bed in North Queensland, Australia", *Pacific Science*, vol. 59, no. 4, pp. 541-560.
- Largier, J.L., Attwood, C. & Harcourt-Baldwin, J.L. 2000, "The hydrographic character of the Knysna Estuary", *Transactions of the Royal Society of South Africa*, vol. 55, pp. 107-122.
- Maree, B. 2000, "Structure and status of the intertidal wetlands of the Knysna Estuary", *Transactions of the Royal Society of South Africa*, vol. 55, pp. 163-176.
- Montague, C.L. & Ley, J.A. 1993, "A possible effect of salinity fluctuation on abundance of benthic vegetation and associated fauna in northeastern Florida Bay", *Estuaries*, vol. 16, no. 4, pp. 703-717.
- Pascual, E. & Drake, P. 2008, "Physiological and behavioral responses of the mud snails *Hydrobia glyca* and *Hydrobia ulvae* to extreme water temperatures and salinities: Implications for their spatial distribution within a system of temperate lagoons", *Physiological and Biochemical Zoology*, vol. 81, no. 5, pp. 594-604.
- Remane, A. 1971, "Ecology of brackish water", *Biology of Brackish Water*, vol. 25, no. PART 1, pp. 1-210.
- Remane, A. 1934, "Die Brackwasserfauna", *Verhandlungen der Deutschen Zoologischen Gesellschaft*, vol. 36, no. SUPPL., pp. 34-74.
- Rodrigues, A.M., Quintino, V., Sampaio, L., Freitas, R. & Neves, R. 2011, "Benthic biodiversity patterns in Ria de Aveiro, Western Portugal: Environmental-biological relationships", *Estuarine, Coastal and Shelf Science*, vol. 95, no. 2-3, pp. 338-348.
- Russell, I.A., Randall, R.M. & Kruger, N. 2009, "Garden Route National Park, Knysna Coastal Section, State of Knowledge", *Garden Route National Park, Knysna Coastal Section, State of Knowledge*, .
- Schlacher, T.A. & Wooldridge, T.H. 1996, "Axial zonation patterns of subtidal macrozoobenthos in the gamtoos estuary, South Africa", *Estuaries*, vol. 19, no. 3, pp. 680-696.
- Short, F.T., Coles, R., Waycott, M., Bujang, J.S., Fortes, M., Prathep, A., Kamal, A.H.M., Jagtap, T.G., Bandeira, S., Freeman, A., Erfmeijer, P., La Nafie, Y.A., Vergara, S., Calumpong, H.P. & Makm, I. *Zostera capensis*. In: *IUCN Red List of Threatened Species, Version 2011.1*, .
- Short, F.T., Polidoro, B., Livingstone, S.R., Carpenter, K.E., Bandeira, S., Bujang, J.S., Calumpong, H.P., Carruthers, T.J.B., Coles, R.G., Dennison, W.C., Erfmeijer, P.L.A., Fortes, M.D., Freeman, A.S., Jagtap, T.G., Kamal, A.H.M., Kendrick, G.A., Judson Kenworthy, W., La Nafie, Y.A., Nasution, I.M., Orth, R.J., Prathep, A., Sanciangco, J.C., Tussenbroek, B.V., Vergara, S.G., Waycott, M. & Zieman, J.C. 2011, "Extinction risk assessment of the world's seagrass species", *Biological Conservation*, vol. 144, no. 7, pp. 1961-1971.
- Stone, L. & Roberts, A. 1990, "The checkerboard score and species distributions", *Oecologia*, vol. 85, no. 1, pp. 74-79.

- Tanner, J.E. 2005, "Edge effects on fauna in fragmented seagrass meadows", *Austral Ecology*, vol. 30, no. 2, pp. 210-218.
- Telesh, I.V. & Khlebovich, V.V. 2010, "Principal processes within the estuarine salinity gradient: A review", *Marine pollution bulletin*, vol. 61, no. 4-6, pp. 149-155.
- Teske, P.R. & Wooldridge, T. 2001, "A comparison of the macrobenthic faunas of permanently open and temporarily open/closed South African estuaries", *Hydrobiologia*, vol. 464, pp. 227-243.
- Teske, P.R. & Wooldridge, T.H. 2004, "Affinities of some common estuarine macroinvertebrates to salinity and sediment type: Empirical data from Eastern Cape estuaries, South Africa", *African Zoology*, vol. 39, no. 2, pp. 183-192.
- Teske, P.R. & Wooldridge, T.H. 2003, "What limits the distribution of subtidal macrobenthos in permanently open and temporarily open/closed South African estuaries? Salinity vs. sediment particle size", *Estuarine, Coastal and Shelf Science*, vol. 57, no. 1-2, pp. 225-238.
- Turpie, J.K. 2004, "South African National Spatial Biodiversity Assessment 2004: Technical Report", *Estuary Component*, vol. 3.
- Turpie, J.K., Adams, J.B., Joubert, A., Harrison, T.D., Colloty, B.M., Maree, R.C., Whitfield, A.K., Wooldridge, T.H., Lamberth, S.J., Taljaard, S. & Van Niekerk, L. 2002, "Assessment of the conservation priority status of South African estuaries for use in management and water allocation", *Water SA*, vol. 28, no. 2, pp. 191-206.
- Turpie, J.K. & Clark, B. 2007, "CAPE Regional Estuarine Management Programme", *Development of a Conservation Plan for Temperate South African Estuaries on the Basis of Biodiversity Importance, Ecosystem Health and Economic Costs and Benefits*, .
- Whitfield, A.K. 1992, "A characterization of southern African estuarine systems", *South African Journal of Aquatic Science*, vol. 18, no. 1-2, pp. 89-103.
- Whitfield, A.K., Elliott, M., Basset, A., Blaber, S.J.M. & West, R.J. 2012, "Paradigms in estuarine ecology - A review of the Remane diagram with a suggested revised model for estuaries", *Estuarine, Coastal and Shelf Science*, vol. 97, pp. 78-90.
- Yamada, K., Hori, M., Tanaka, Y., Hasegawa, N. & Nakaoka, M. 2007, "Temporal and spatial macrofaunal community changes along a salinity gradient in seagrass meadows of Akkeshi-ko estuary and Akkeshi Bay, northern Japan", *Hydrobiologia*, vol. 592, no. 1, pp. 345-358.