Estimating macrozoobenthic species richness along an environmental gradient: Sample size matters

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ABSTRACT

Along monotonous environmental gradients (such as increasing temperatures, salinities, heights, sediment coarseness, etc), numbers of species per unit area can change in consistent ways, either in a monotonous or a more complex way (e.g. showing a peak somewhere along the gradient). We studied patterns of species density in bivalves in a Wadden Sea tidal flat area from a data set obtained by long-term monitoring at numerous sampling stations at various distances (0–10 km) from the shore line. At short distances from the shores, tidal flats are generally high and sheltered from strong winds and tidal currents and sediments are muddy. With increasing distance from the shore, both intertidal level as well as shelter tends to decline, resulting in declining trends in mud content and increasing trends in median grain size of the sediments. Species numbers in samples of 0.1 and 1 m^2 were found to decline monotonously with increasing distance from the shore. However, in large aggregated samples (38 m^2), maximal species numbers (approaching real species richness) were not found close to the shore, but had shifted into an off-shore direction. As a consequence, differential multiplication factors had to be used to obtain an asymptotic estimate of total species richness from actually observed figures of species numbers in small samples that were taken in different areas: for samples of 0.1 m^2 (1 m^2) these factors amounted to ~3 (~2) in near-shore areas and to ~6 (~2.5) in off-shore areas. Species accumulation curves were differentially shaped in near-shore and off-shore areas and intersected, allowing a reliable estimate of assemblage species richness in an area by extrapolation only when large aggregated samples (of well over 1 m^2) were available. The base of these differences was a differential distribution of abundant and rare species. The few species with numerical densities frequently exceeding 100 individuals m^{-2} occurred particularly in near-shore areas, whereas the more numerous low-density species (<10 individuals m^{-2}) were more frequently observed in off-shore areas. Because small samples adequately catch only the abundant species and almost completely miss rare species, they show little more than the locations where abundant species occur. An unambiguous answer to the question "where along a gradient is species density maximal" cannot be given, as it depends on sample size. Certainly on tidal flats, and may be in other heterogeneous benthic areas, too small samples give a false impression of the location of hotspots of species richness.

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1. Introduction

Gradients of environmental factors and species richness are omnipresent at all scales, from worldwide latitudinal (Gaston, 2000) to micro-scales. The present paper deals with relatively fine-scaled patterns of changes in environmental factors and species density along a gradient of some 10 km perpendicular to the shore line in a soft-bottom intertidal area.

Observed patterns of change of species richness along environmental gradients are far from universal. They may be either unidirectional (such as the well known increasing species richness from the poles towards the tropics: Gaston, 2000; Lomolino et al., 2010) or of a more complex nature, e.g. showing a humpback (unimodal) shape with a maximal richness at some place at the gradient (e.g. the observation in various invertebrates groups of maximal species numbers at intermediate depths of continental slopes: Lomolino et al., 2010) or otherwise. According to Nogués-Bravo et al. (2008), the obtained type of pattern may depend on the methods used, being more sensitive to scale (i.e. length of the gradient section) than to grain size (surface area of single sampling

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unit). In the present study, we are particularly interested in the possible influence of sampling grain size (range of size of individual samples, from 0.1 to 38 m^2) on the pattern of species richness along an environmental gradient and on the location of maximal diversity.

Patterns in species density (number of species per unit area) can be assessed by taking samples at equal distances along the gradient. Generally, such samples will be too small to contain all or nearly all species present. They must be aggregated to allow a fair estimate of real species richness. The usual way to estimate species richness of an area is by construction of a species accumulation curve: plotting the increasing species number with increasing surface area sampled reveals by extrapolation an asymptotic value of the species number at very large sample sizes. If these total areas sampled are too small to include a substantial portion of the species number actually present, the asymptotic value may be a poor representation of the true species richness (Gotelli and Colwell, 2001). Various extrapolation methods are available and have been tested to estimate assemblage species richness ("γ diversity") from species numbers encountered in individual samples ("α diversity"), in the marine realm for instance by Foggo et al. (2003), Schoeman et al. (2008), and Canning-Clode et al. (2008). Unfortunately, most extrapolation techniques were found to yield serious underestimates of the "true" species richness (Brose et al., 2003; Canning-Clode et al., 2008), in marine areas most obviously so in the soft-bottom environment (Canning-Clode et al., 2008). This bias invariably declined with increasing sampling effort (Foggo et al., 2003; Schoeman et al., 2008; Canning-Clode et al., 2008), raising the question how large samples should be to predict with sufficient precision the "true" species richness.

Species accumulation curves show increases at a decelerating rate to an asymptotic value that may be considered as the "true" species richness. However, the exact shape of such accumulation curves may differ in different areas, making the unavoidable bias in the extrapolated value of species richness unpredictable and probably different for different areas. For instance: if the assemblage is made up almost exclusively by species with high numerical densities, the use of small samples may suffice to estimate total species number (because nearly all species are already represented in small samples and larger samples would hardly add new species to the list). On the other hand, in the case of an area that is inhabited by many species of low abundance, large samples would be needed to encounter a sufficient proportion of the total assemblage, i.e. to reach sufficient "coverage" (Brose et al., 2003). When most species are present in low numbers, the initial part of the species accumulation curve is bound to rise slowly with increasing sample size and a wide range of samples sizes (meaning a high sampling effort) would be needed to reliably construct a species accumulation curve and estimate the total species richness of the assemblage. Thus, when species richness of two or more areas is to be compared, these areas may not necessarily have the same shape of the species accumulation curve. The curves for the two areas may even intersect (Gotelli and Colwell, 2001), meaning that small and large samples provide opposite answers to the question of which one of the two compared areas is the richest in species. Therefore, the first thing to do is studying the relationships between sample size and species richness in the areas to be compared.

The availability of data of a long-term and large-scale monitoring program of macrobenthic animals enabled us to study this relationship in a Wadden Sea tidal-flat area. We arranged the 15 sampling sites in the order of increasing distance from the shore, which reflected gradients from sheltered to exposed conditions (going with increasing coarseness of the sediments) and from high to low intertidal heights (with increasing daily periods of

inundation). The pattern of change of species density along this gradient was assessed for samples of a wide range of sizes: from ~0.1 to pooled samples of ~1, ~10 and ~38 m^2.

Earlier studies of Wadden Sea macrozoobenthos ((Beukema, 1976, 1988; Dankers and Beukema, 1983: their figs 11 and 12; Armonies and Hellwig-Armonies, 1987) revealed that species numbers of benthic animals are maximal at intermediate rather than at either very fine or very coarse sediments. For individual species, sediment/occurrence relationships showed a wide variety of curve shapes, only part of them revealing maximal probability of occurrence at intermediate sediment composition (Ysebaert et al., 2002; Thrush et al., 2003; Kraan et al., 2010). However, summed numbers of species with a certain minimal probability of occurrence at various sediment characteristics would invariably yield maximal species numbers at intermediate values of mud content or median grain size (compare figure 6 of Ysebaert et al., 2002 and figure 3 of Thrush et al., 2003). Thus, species richness optima at intermediate values of sediments characteristics may be the rule in marine macrozoobenthos. Notable exceptions, however, were reported by Compton et al. (2008), who found that bivalve species numbers per sample consistently decline with increasing coarseness of the sediment. Their finding of similar relationship of this shape in a number of different soft-sediment areas around the world (including tidal flats in the Wadden Sea) suggests a generally occurring relationship. Moreover, a similarly shaped relationship was observed by Van Colen et al. (2010) for deposit feeding species in an intertidal estuarine soft-bottom area. The question rises whether the above results of species number maxima at intermediate versus fine sediments are really contradictory or can be attributed to methodological differences.

An obvious difference in methodology between the above studies is applied sample size. The results reported by Compton et al. (2008) were based on single samples each covering only 0.02 m^2 and, therefore, called "point diversity". As a consequence, their counts of species numbers ranged from a mean value of mostly 1 or 2 in samples taken in the finest (muddy) sediments to <1 in the coarser sands, being an order of magnitude lower than bivalve species numbers found in large samples taken in such areas. The few species they found per sample may have been a non-typical part of the fauna present, for instance by an underrepresentation of species occurring in low numbers which would have negligible chances to be collected in small samples.

The present study aims: (1) to find out which pattern of species density is characteristic along a gradient on tidal flats perpendicular to the shore; (2) to explore whether extrapolations of species numbers with increasing sample size differ at different environmental conditions and to judge in this way what minimal sample size would provide a reliable estimate of assemblage species richness, and (3) to explain why the (monotonous) relationships between sediment characteristics and species numbers per sample as found by Compton et al. (2008) and Van Colen et al. (2010) showed a shape that differed from the (humpback) relationships as reported by Beukema (1976, 1988), Dankers and Beukema (1983) and Armonies and Hellwig-Armonies (1987).

2. Methods

2.1. Data collection

Since *ca.*1970, data on macrozoobenthos have been collected twice annually for 40 years at 15 permanent (marked) sampling stations ("sites") located on Balgzand, a 50-km^2 tidal flat area in the westernmost part of the Wadden Sea (Fig. 1). In the present study, we use data collected in late-winter/early spring, when we took larger samples than in summer, because in winter numbers of