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The accuracy of density standardization of infaunal benthos

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In benthic intertidal studies it is common to standardize species density, and other variables, to a square metre, regardless of the surface area sampled. Using a fully-nested sampling design, with sampling unit surface areas ranging from 0.020 to 1 m^2, the present study investigated the effect of this procedure on the accuracy of the results. It is demonstrated that this standardization has a profound influence on the resultant estimates, introducing a substantial error into inter-study comparisons.

The two aspects of sampling design that most affect the accuracy and precision of density estimates are the size of the sampling unit and the number of replicates, particularly the relationship between size of sampling units and variance of estimated densities (e.g. Downing, 1989). Nevertheless, the majority of benthic studies employ a sample size dictated by custom and tradition (Andrews & Mapstone, 1987). As these have not been critically evaluated, a range of sizes have been used, ranging from 0.0028 m^2 (e.g. Frid & James, 1989) to 1 m^2 (e.g. Miron & Desrosiers, 1990).

It is necessary to express abundance and derived parameters as a standardized surface area, traditionally 1 m^2. After such standardization, it appears to be universally accepted these estimates can be compared with previous studies. For example, Nithart (1998) compared density, biomass and production values (expressed per m^2) of *Nereis diversicolor* with 18 other studies, which employed sampling unit sizes from 0.0025 m^2 to 0.25 m^2.

The study site was a semi-exposed, sandy beach in Ringaskiddy (Cork Harbour, Ireland); tidal range 3.3 m, medium-coarse sand substrate, low percentage of organic matter. Samples were taken at ~0.6 m above Chart Datum. A fully-nested sampling design (illustrated by Ballesteros, 1986) was used, with the following surface areas sampled: 0.0020 m^2, 0.0060 m^2, 0.0137 m^2, 0.0292 m^2, 0.0605 m^2, 0.1230 m^2, 0.2402 m^2, 0.4746 m^2, 0.8790 m^2 and 1 m^2. In this design, each corer is nested within the next larger size one, and so on up to the largest corer, essentially a Russian doll design. Exact positions of each corer within the next larger one were randomized as far as possible throughout the replication, however, size restrictions did not allow for a fully randomized design. Sampling units consisted of square box corers driven into the sediment to a depth of 20 cm. Ten replicate sets of nested samples were taken, sieved in small batches on a 0.5 mm sieve and sorted by eye.

For comparison a further set of six replicate, nested-sampling units were obtained from a nearby muddy area, with similar tidal characteristics.

As the variance was not equal across the sampling units and because of the non-independence of the observations, no formal statistical testing could be carried out on the data. For graphical purposes, the density obtained per sampling unit, was multiplied up with the appropriate factor in order to be expressed as 'estimated density' of each species per m^2. The term 'true 1 m^2 density' is here restricted to the mean density value of the 1 m^2 sampling units. Zero returns were included in the data set.

A total of 12 species was encountered in the sandy data set, numerically dominated by *Tellina tenuis* and *Nephtys hombergii*. When comparing the 'estimated density' of any given sampling unit to the 'true 1 m^2 density', it was obvious that the majority of sampling units over-estimated the 'true 1 m^2 density' (Figures 1 & 2). As expected, this over-estimation diminished as the surface area of the sampling unit approaches the 1 m^2 mark. Conversely, the largest over-estimation was always seen in the smallest sampling units, in which any given species is present. It is also clear that the amount of over-estimation depended on the 'true 1 m^2 density' of the species, with high-density species being less over-estimated in smaller sampling units than low-density species. For instance, species such as *T. tenuis* and *N. hombergii* were only over-estimated by a factor of 1.67 and 2.86 in the smallest sampling unit in which they were present, whilst this ratio is 28.51 for *Eumida sanguinea* (Oersted) and 60.95 for *Bathyporeia guilliamsoniana* (Bate) (Figure 1).

For the two high-density species, the larger and medium-sized (the latter only for *T. tenuis*) sampling units, over-estimated the 'true 1 m^2 density' by a factor of less than 1.5. In fact, for *T. tenuis* only the smallest sampling unit provided a mean over-estimation of more than 1.5. However, the maximum–minimum ratio is quite large in medium to small sampling units. In the low-density species, a similar trend is observed, however much higher values of both the mean and the maximum–minimum ratio were observed.

To investigate whether the observed trends were unique to the sand data set, two species were enumerated from the muddy data set: *N. hombergii* and *Nereis diversicolor* (Figure 2). Clearly, the same trends are observed; however, when comparing the pattern observed in both data sets for *Nephtys hombergii*, some differences were apparent (Figures 1 & 2). The maximum–minimum ratio of a given sampling unit is much larger on sand than on mud. This is consistent with the observations of Martin et al. (1993) who observed that the patch size of estuarine polychaetes in muddy environments were smaller than under sandy conditions. Therefore, a sampling unit surface area larger than the aggregation pattern of *N. hombergii* in mud will produce less variable results than if the same corer is used on the more dispersed sand populations.

The present study clearly demonstrated that the practice of standardization of density estimates to a 1 m^2 should be treated with considerable scepticism and indeed is a practice which is better avoided in future studies. Considerable caution is advised when comparing density estimates with other studies, especially if the surface area of the sampling units differ greatly from each other. The spatial patchiness of the species is undoubtedly responsible for the observed pattern, both in terms of patch size and within-patch densities. This is most clearly observed in the number of zero returns, which increases as sampling unit surface area decreases.

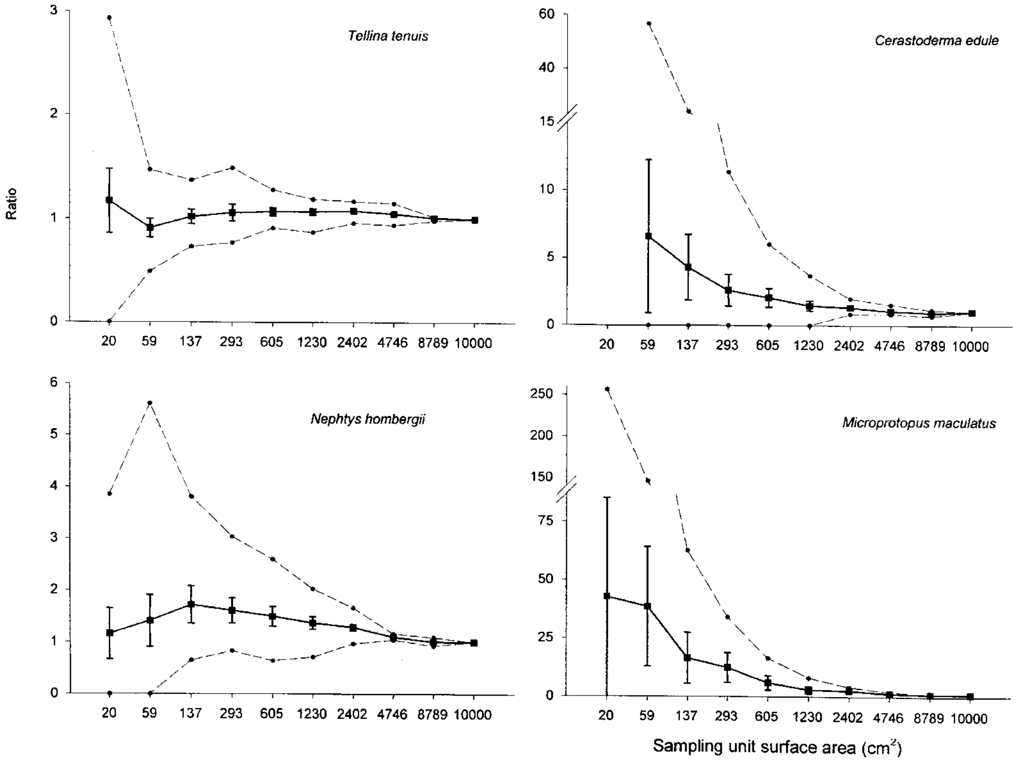

Figure 1. Ratio of estimated density to 'true density' for *Tellina tenuis*, *Nephtys hombergii*, *Cerastoderma edule* and *Microprotopus maculatus* from sand data set. Bold line indicates mean values and standard error, dashed lines indicate maxima and minima.

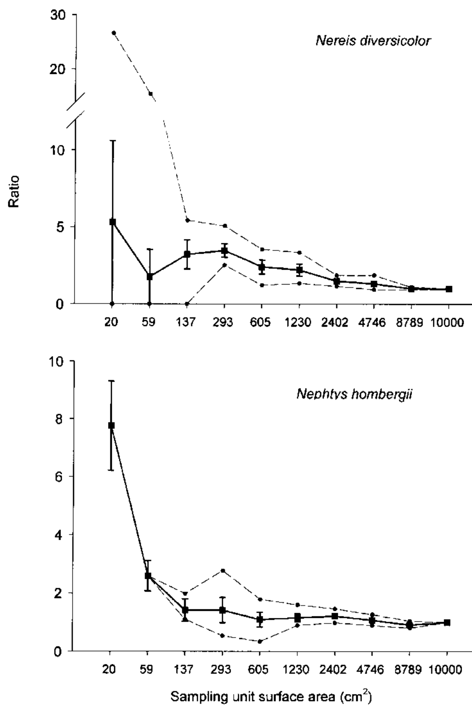

Figure 2. Ratio of estimated density to 'true density' for *Nereis diversicolor* (top) and *Nephtys hombergii* (bottom) from mud data set. For explanation, see Figure 1.

Certain types of benthic studies would benefit from a 'standard surface area' being used in sampling, similar to the near-universal use of the 0.25 m^2 quadrat in rocky shore studies and 0.1 m^2 in subtidal benthic studies. Based on the present study (admittedly limited in scope), a sampling unit surface area of at least 0.1230 m^2 should be deployed in sandy habitats, and 0.0137 m^2 in muddy habitats. This would in particular benefit studies such as community mapping, impact assessment, and other areas in which scientists are often asked to provide managerial and environmental decisions which by necessity require a comparison with other data sets.

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